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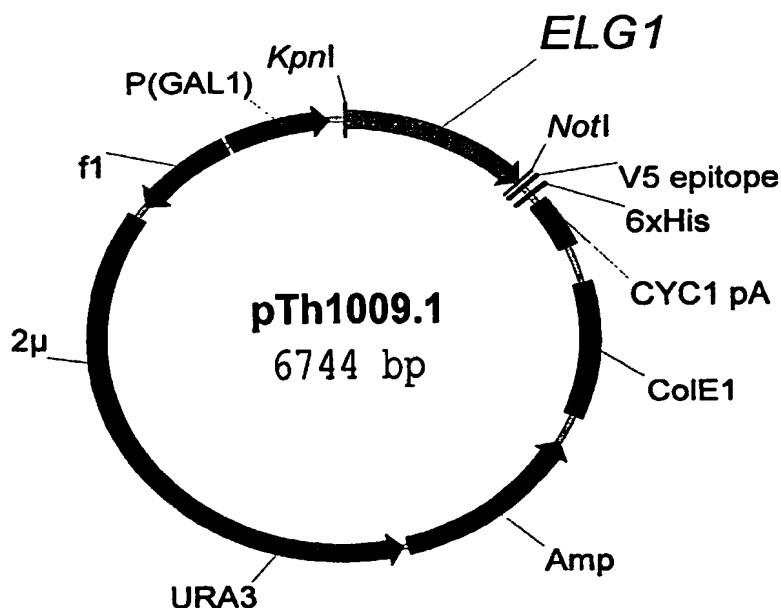
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(54) Title: **HUMAN ELONGASE GENES USES THEREOF AND COMPOUNDS FOR MODULATING SAME**



(57) Abstract: The present invention relates to elongase genes, their polypeptides and their control regions, and the use of such genes, polypeptides and control regions in determining compositions for use in the treatment of disease. The identified compositions regulate the expression of the elongase genes or modulate the activity of their protein products. The nucleotide and amino acid sequences are taught for ELG4, ELG6 and ELG7. The control sequences and function are taught for ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7.



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## Human Elongase Genes, Uses Thereof, and Compounds for Modulating Same

### FIELD OF THE INVENTION

- 5 This invention relates to the identification of compounds that modulate the activity of fatty acid elongase enzymes involved in lipid metabolism and/or effectively regulate the level of expression of the elongase genes, and to compounds so identified.

### 10 BACKGROUND OF THE INVENTION

Polyunsaturated fatty acids (PUFAs) are major components of lipid compounds and complexes, such as phospholipids and lipoproteins, which provide a number of structural and functional characteristics to a wide range of biological constituents, such as the cell  
15 membranes. PUFAs are essential for the proper development, maintenance and repair of tissue. Other biological functions of PUFAs include their involvement in the expression of some genes and their role as precursor molecules for conversion into biologically active metabolites that regulate critical physiological functions. Consequently, a lack of, or imbalance in, PUFA levels has been attributed to certain pathological conditions.

20 Figures 1, 2 and 3 show the required desaturation and elongation steps for the production of long chain fatty acids in the n-3, n-6 and n-9/n-7 PUFA families, respectively. Fatty acid chain elongation systems have been found in liver, brain, kidney, lung, adrenals, retina, testis, small intestine and blood cells, namely leukocytes (Cinti et al., 1992, *Prog. Lipid Res.*, 31: 1-51).

25 Elongase genes have been identified in *Arabidopsis* (James et al., 1995, *Plant Cell*, 7: 309-319) and in *C. elegans* (WO 00/55330, Sept., 2000, Napier J.A.). Three separate elongase genes, ELO1, ELO2 and ELO3, have been identified from *S. cerevisiae*. ELO1 elongates myristic acid to palmitic acid (Toke D.A. and Martin C.E., 1996, *J. Biol. Chem.*, 271: 18413-18422) while ELO2 and ELO3 elongate long chain saturated fatty acids (Oh et al., 1997, *J. Biol. Chem.*, 272: 17376-17384).

Deficiencies in polyunsaturated fatty acids (PUFAs) have been associated with a number of diseases such as eczema, cardiovascular disorders, inflammation, psychiatric disorders, cancer, cystic fibrosis, pre-menstrual syndrome and diabetes (Horrobin D.F. [ed.], 1990, *Omega-6 Essential Fatty Acids: Pathophysiology and Roles in Clinical Medicine*, Wiley-Liss, NY and  
30 Mazza G. and Domah B.D. [eds.], 2000, *Herbs, Botanicals and Teas*, Technomic Publishers,

Lancaster, PA). Diets supplemented with PUFAs have been attempted as a treatment for a number of these conditions. The level of success for such applications has varied considerably.

- 5 Low levels of linoleic acid (18:2n-6, LA), dihomogamma-linolenic acid (20:3n-6, DGLA) and arachidonic acid (20:4n-6, AA) in adipose tissue of males have been correlated with increased mortality from coronary heart disease (Riemersma et al., 1986, *Br. Med. J. [Clin. Res. Ed.]*, 292: 1423-1427). The supplementation of LA and alpha-linolenic acid (18:3n-3, ALA) to patients suffering from hypertension did not increase the tissue levels of AA or eicosapentaenoic acid (20:5n-3, EPA) which indicates defective desaturation and elongation in
- 10 the n-6 and n-3 fatty acid systems (Singer et al., 1984, *Prostaglandins Leukot. Med.*, 15: 159-165). Misoprostol, a prostaglandin E1 (PGE1) analogue, has been successfully used to treat peripheral vascular disease (Goszcz et al., 1998, *Methods Find. Exp. Clin. Pharmacol.*, 20: 439-445). PGE1 is a cyclooxygenase product of DGLA.
- 15 It has been observed that PUFAs can alleviate and correct some of the symptoms of diabetic neuropathy (Dines et al., 1993, *Diabetologia*, 36: 1132-1138 and Cotter et al., 1995, *Diabetic Neuropathy: New Concepts and Insights*, Elsevier Science B.V., Amsterdam, pp. 115-120). Researchers have speculated that the production or modulation of the cyclooxygenase and lipoxygenase metabolites of the n-3 and n-6 fatty acid families is responsible for some of these
- 20 beneficial effects.

Most of the lipid metabolism disorders are characterized by a deficiency in essential fatty acids. This deficiency has been attributed to altered rate-limiting steps of delta-6-desaturation (D6D) and/or delta-5-desaturation (D5D) in PUFA biosynthesis.

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### SUMMARY OF INVENTION

- The present invention teaches an isolated polynucleotide sequence, comprising a
- 30 polynucleotide sequence which is selected from the group consisting of: (a) a sequence comprising SEQ ID NO: 4 (ELG4); (b) a sequence comprising SEQ ID NO: 8 (ELG6); (c) a sequence comprising SEQ ID NO: 11 (ELG7); (d) a sequence which is at least 80% homologous with a sequence of any of (a) to (c); (e) a sequence which is at least 90% homologous with a sequence of any of (a) to (c); (f) a sequence which is at least 95%
- 35 homologous with a sequence of any of (a) to (c); (g) a sequence which is at least 98% homologous with a sequence of any of (a) to (c); (h) a sequence which is at least 99%

homologous with a sequence of any of (a) to (c); and; (i) a sequence which hybridizes to any of (a) to (h) under stringent conditions. The isolated polynucleotide sequence may be cDNA.

5 The invention also teaches an isolated polypeptide comprising an isolated polypeptide selected from the group consisting of: (a) a sequence comprising SEQ ID NO: 5 (ELG4); (b) a sequence comprising SEQ ID NO: 9 (ELG6); (c) a sequence comprising SEQ ID NO: 12 (ELG7); (d) a sequence which is at least 80% homologous with a sequence of any of (a) to (c); (e) a sequence which is at least 90% homologous with a sequence of any of (a) to (c); (f) a sequence which is at least 95% homologous with a sequence of any of (a) to (c); (g) a  
10 sequence which is at least 98% homologous with a sequence of any of (a) to (c); and (h) a sequence which is at least 99% homologous with a sequence of any of (a) to (c).

The invention teaches an isolated polynucleotide sequence, comprising a polynucleotide sequence which is selected from the group consisting of: (a) a sequence comprising SEQ ID  
15 NO: 1 (control region for ELG1); (b) a sequence comprising SEQ ID NO: 2 (control region for ELG2); (c) a sequence comprising SEQ ID NO: 3 (control region for ELG3); (d) a sequence comprising SEQ ID NO: 6 (control region for ELG4); (e) a sequence comprising SEQ ID NO: 7 (control region for ELG5); (f) a sequence comprising SEQ ID NO: 10 (control region for ELG6); (g) a sequence comprising SEQ ID NO: 13 (control region for ELG7); (h)  
20 a sequence which is at least 80% homologous with a sequence of any of (a) to (g); (i) a sequence which is at least 90% homologous with a sequence of any of (a) to (g); (j) a sequence which is at least 95% homologous with a sequence of any of (a) to (g); (k) a sequence which is at least 98% homologous with a sequence of any of (a) to (g); (l) a sequence which is at least 99% homologous with a sequence of any of (a) to (g); and;  
25 (m) a sequence which hybridizes to any of (a) to (l) under stringent conditions.

The invention includes an isolated polynucleotide fragment selected from the group consisting of: (a) a sequence having at least 15 sequential bases of nucleotides of a sequence of the invention; (b) a sequence having at least 30 sequential bases of nucleotides of a sequence of  
30 the invention; and (c) a sequence having at least 50 sequential bases of nucleotides of a sequence of the invention. The invention includes a polypeptide sequence which retains substantially the same biological function or activity as or is a functional derivative of a polypeptide sequence of the invention.

35 The invention includes an isolated polynucleotide sequence, comprising a polynucleotide sequence which retains substantially the same biological function or activity as or is a functional derivative of a polynucleotide sequence of the invention.

The invention also teaches a vector comprising a polynucleotide sequence of the invention in a suitable vector. The vector may be heterologous to the sequence. The vector may contain or encode a tag. The invention also teaches a host cell comprising a polynucleotide sequence of the invention in a host cell which is heterologous to the sequence.

The invention teaches a method for identifying a compound which inhibits or promotes the activity of a polynucleotide sequence of the invention, comprising the steps of: (a) selecting a control animal having the sequence and a test animal having the sequence; (b) treating the test animal using a compound; and, (c) determining the relative quantity of an expression product of the sequence, as between the control animal and the test animal.

The invention also teaches a method for identifying a compound which inhibits or promotes the activity of a polynucleotide sequence of the invention, comprising the steps of: (a) selecting a host cell of the invention; (b) cloning the host cell and separating the clones into a test group and a control group; (c) treating the test group using a compound; and (d) determining the relative quantity of an expression product of the sequence, as between the test group and the control group.

The invention further teaches a method for identifying a compound which inhibits or promotes the activity of a polynucleotide sequence of the invention, comprising the steps of: (a) selecting a test group having a host cell of the invention or a part thereof, and selecting a suitable control group; (b) treating the test group using a compound; and (c) determining the relative quantity or relative activity of a product of the sequence or of the sequence, as between the test group and the control group.

The invention teaches a process for producing a polypeptide sequence of the invention comprising the step of culturing the host cell of the invention under conditions sufficient for the production of the polypeptide.

The invention teaches a method for identifying a compound which inhibits or promotes the activity of a polypeptide sequence of the invention, comprising the steps of: (a) selecting a control animal having the sequence and a test animal having the sequence; (b) treating the test animal using a compound; (c) determining the relative quantity or relative activity of an expression product of the sequence or of the sequence, as between the control animal and the test animal.



The invention also teaches a method for identifying a compound which inhibits or promotes the activity of a polypeptide sequence of the invention, comprising the steps of: (a) selecting a host cell of the invention; (b) cloning the host cell and separating the clones into a test group and a control group; (c) treating the test group using a compound; and (d) determining the relative quantity or relative activity of an expression product of the sequence or of the sequence, as between the test group and the control group.

The invention includes a method for identifying a compound which inhibits or promotes the activity of a polypeptide sequence of the invention, comprising the steps of: (a) selecting a test group having a host cell of the invention or a part thereof, and selecting a suitable control group; (b) treating the test group using a compound; and (c) determining the relative quantity or relative activity of a product of the sequence or of the sequence, as between the test group and the control group.

The invention includes a method for identifying a compound which modulates a biological activity of a polypeptide sequence of the invention, comprising the steps of: (a) providing an assay which measures a biological activity of a polypeptide sequence of the invention; (b) treating the assay with a compound; and (c) identifying a change in the biological activity of the polypeptide, wherein a difference between the treated assay and a control assay identifies the compound as modulator of the polypeptide. The polypeptide in this assay may be provided in a purified, reconstituted, cell extract or whole cell assay format, as required to assay the biological activity in question.

The invention also teaches a method for identifying a compound which inhibits or promotes the activity of a polynucleotide sequence of the invention, comprising the steps of: (a) selecting a host cell of the invention; (b) cloning the host cell and separating the clones into a test group and a control group; (c) treating the test group using a compound; and (d) determining the relative quantity of an expression product of an operably linked polynucleotide to the sequence, as between the test group and the control group.

The invention also teaches a method for identifying a compound which inhibits or promotes the activity of a polynucleotide sequence of the invention, comprising the steps of: (a) selecting a test group having a host cell of the invention or a part thereof, and selecting a suitable control group; (b) treating the test group using a compound; and (c) determining the relative quantity of an expression product of an operably linked polynucleotide to the sequence, as between the test group and the control group.

The invention includes a composition for treating a PUFA disorder comprising a compound which modulates a sequence of the invention and a pharmaceutically acceptable carrier. The invention includes the use of a composition of the invention for treating PUFA disorders.

- 5 The invention includes a method for diagnosing the presence of or a predisposition for a PUFA disorder in a subject by detecting a germline alteration in a sequence of the invention in the subject, comprising comparing the germline sequence of a sequence of the invention from a tissue sample from the subject with the germline sequence of a wild-type of the sequence, wherein an alteration in the germline sequence of the subject indicates the presence of or a
- 10 predisposition to the PUFA disorder. The invention teaches a method for diagnosing the presence of or a predisposition for a PUFA disorder in a subject, comprising comparing the sequence of a polypeptide of the invention from a tissue sample from the subject with the sequence of a wild-type of the polypeptide, wherein an alteration in the sequence of the subject as compared to the wild-type indicates the presence of or a predisposition to the PUFA
- 15 disorder.

- The invention also teaches a method for identifying a compound which modulates a PUFA disorder, comprising identifying a compound which modulates the activity of a polynucleotide, wherein the polynucleotide is a coding sequence selected from the group
- 20 consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7, comprising the steps of: (a) selecting a control animal having the polynucleotide and a test animal having the polynucleotide; (b) treating the test animal using a compound; and, (c) determining the relative quantity of an expression product of the polynucleotide, as between the control animal and the test animal.

- 25 The invention further teaches a method for identifying a compound which modulates a PUFA disorder, comprising identifying a compound which modulates the activity of a polynucleotide, wherein the polynucleotide is a coding sequence selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7, comprising the steps of:
- 30 (a) selecting a host cell having the polynucleotide, wherein the host cell is heterologous to the polynucleotide; (b) cloning the host cell and separating the clones into a test group and a control group; (c) treating the test group using a compound; and (d) determining the relative quantity of an expression product of the polynucleotide, as between the test group and the control group.

- 35 The invention further teaches a method for identifying a compound which modulates a PUFA disorder, comprising identifying a compound which modulates the activity of a

polynucleotide, wherein the polynucleotide is a coding sequence selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7, comprising the steps of: (a) selecting a test group having a host cell with the polynucleotide or a portion of the host cell, and selecting a suitable control group; (b) treating the test group using a compound; and  
5 (c) determining the relative quantity or relative activity of a product of the polynucleotide or of the polynucleotide, as between the test group and the control group.

The invention teaches a method for identifying a compound modulates a PUFA disorder, comprising identifying a compound which modulates the activity of a polypeptide selected  
10 from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7, comprising the steps of: (a) selecting a control animal having the polypeptide and a test animal having the polypeptide; (b) treating the test animal using a compound; (c) determining the relative quantity or relative activity of an expression product of the polypeptide or of the polypeptide, as between the control animal and the test animal.

The invention further teaches a method for identifying a compound which modulates a PUFA disorder, comprising identifying a compound which modulates the activity of a polypeptide selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7, comprising the steps of: (a) selecting a host cell comprising the polypeptide, wherein the host  
20 cell is heterologous to the polypeptide; (b) cloning the host cell and separating the clones into a test group and a control group; (c) treating the test group using a compound; and (d) determining the relative quantity or relative activity of an expression product of the polypeptide or of the polypeptide, as between the test group and the control group.

The invention also teaches a method for identifying a compound which modulates a PUFA disorder, comprising identifying a compound which modulates the activity of a polypeptide selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7, comprising the steps of: (a) selecting a test group having a host cell with the polynucleotide or a portion of the host cell, and selecting a suitable control group; (b) treating the test group  
30 using a compound; and (c) determining the relative quantity or relative activity of a product of the polypeptide or of the polypeptide, as between the test group and the control group.

The invention further teaches a method for identifying a compound which modulates the activity of a polynucleotide, wherein the polynucleotide is a control region of a gene selected  
35 from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7, comprising the steps of: (a) selecting a control animal having the polynucleotide and a test animal having the polynucleotide; (b) treating the test animal using a compound; and, (c)

determining the relative quantity of an expression product of an operably linked polynucleotide to the polynucleotide, as between the control animal and the test animal.

The animals of the invention may be mammals. The mammals may be rats.

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The invention also teaches a method for identifying a compound which modulates the activity of a polynucleotide, wherein the polynucleotide is a control region of a gene selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7, comprising the steps of: (a) selecting a host cell comprising the polynucleotide, wherein the host cell is heterologous to the polynucleotide; (b) cloning the host cell and separating the clones into a test group and a control group; (c) treating the test group using a compound; and (d) determining the relative quantity of an expression product of an operably linked polynucleotide to the polynucleotide, as between the test group and the control group.

10

The invention further teaches a method for identifying a compound which modulates the activity of a polynucleotide, wherein the polynucleotide is a control region of a gene selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7, comprising the steps of: (a) selecting a test group having a host cell with the polynucleotide or a portion of the host cell, and selecting a suitable control group; (b) treating the test group using a compound; and (c) determining the relative quantity of an expression product of an operably linked polynucleotide to the polynucleotide, as between the test group and the control group.

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The invention includes a composition for treating a PUFA disorder comprising a compound which modulates a polynucleotide from the coding sequence selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6, and ELG7, and a pharmaceutically acceptable carrier.

25

The invention further teaches a composition for treating a PUFA disorder comprising a compound which modulates a polypeptide selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6, and ELG7, and a pharmaceutically acceptable carrier.

30

The invention further teaches a composition for treating a PUFA disorder comprising a compound which modulates a polynucleotide from the control region selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6, and ELG7, and a pharmaceutically acceptable carrier.

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The compound may be selected from the group consisting of antibodies against ELG1, ELG2, ELG3 and ELG5.

5 The invention includes method for diagnosing the presence of or a predisposition for a PUFA disorder in a subject by detecting a germline alteration in a polynucleotide representing the coding sequence selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6, and ELG7, from the subject, comprising comparing the germline sequence of the polynucleotide from a tissue sample from the subject with the germline sequence of a wild-type of the polynucleotide, wherein an alteration in the germline sequence of the subject  
10 indicates the presence of or a predisposition to the PUFA disorder.

The invention also teaches method for diagnosing the presence of or a predisposition for a PUFA disorder in a subject by detecting a germline alteration in a polynucleotide representing the control region selected from the group consisting of ELG1, ELG2, ELG3 and ELG5 in the  
15 subject, comprising comparing the germline sequence of the polynucleotide from a tissue sample from the subject with the germline sequence of a wild-type of the polynucleotide, wherein an alteration in the germline sequence of the subject indicates the presence of or a predisposition to the PUFA disorder.

20 The invention also teaches a method for diagnosing the presence of or a predisposition for a PUFA disorder in a subject, comprising comparing the sequence of a polypeptide selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6, and ELG7, from the subject with the sequence of a wild-type of the polypeptide, wherein an alteration in the sequence of the subject as compared to the wild-type indicates the presence of or a  
25 predisposition to the PUFA disorder.

The invention further teaches a method for identifying a compound which inhibits or promotes the overall activity of two or more polynucleotides, wherein the polynucleotides are control regions of two or more different genes selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7, comprising the steps of: (a) selecting a host cell  
30 having the polynucleotides, wherein the host cell is heterologous to the polynucleotides; (b) cloning the host cell and separating the clones into a test group and a control group; (c) treating the test group using a compound; and (d) determining the relative quantities of expression products of operably linked polynucleotides to the polynucleotides, as between the  
35 test group and the control group.

- The invention further teaches a method for identifying a compound which inhibits or promotes the overall activity of two or more polynucleotides, wherein the polynucleotides are from control regions of the polynucleotides, selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7, comprising the steps of: (a) selecting a test group  
5 having a host cell with the polynucleotide or a portion of the host cell, and selecting a suitable control group; (b) treating the test group using a compound; and (c) determining the relative quantities of expression products of operably linked polynucleotides to the polynucleotides, as between the test group and the control group.
- 10 The invention teaches a method for identifying a compound which inhibits or promotes the activity of two or more polynucleotides, wherein the polynucleotides are coding sequences selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7, operably associated with promoter regions, wherein the promoter regions are effective to  
15 initiate, terminate or regulate the level of expression of the nucleic acid sequence, comprising the steps of: (a) selecting a host cell having the polynucleotides, wherein the host cell are heterologous to the polynucleotides; (b) cloning the host cell and separating the clones into a test group and a control group; (c) treating the test group using a compound; and (d) determining the relative quantity or relative activity of an expression product of the polynucleotide, as between the test group and the control group.
- 20 The invention further teaches a method for identifying a compound which inhibits or promotes the activity of two or more polynucleotides, wherein the polynucleotides are coding sequences selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7, operably associated with promoter regions, wherein the promoter regions are effective to  
25 initiate, terminate or regulate the level of expression of the nucleic acid sequence, comprising the steps of: (a) selecting a test group having a host cell with the polynucleotide or a portion of the host cell, and selecting a suitable control group; (b) treating the test group using a compound; and (c) determining the relative quantity or relative activity of an expression product of the polynucleotide, as between the test group and the control group.
- 30 The invention includes a method for identifying a compound which inhibits or promotes the activity of a mammalian delta-5-desaturase enzyme and one or more enzymes selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 or ELG7, within the same host system, comprising the steps of: (a) providing a host system containing nucleic acid  
35 sequences which encode for a mammalian delta-5-desaturase and one or more mammalian elongase enzymes selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 or ELG7, operably associated with promoter regions, wherein the promoter regions are

effective to initiate, terminate or regulate the level of expression of the nucleic acid sequence; (b) contacting the host system with a test component; (c) simultaneously evaluating the enzymatic activities of the delta-5-desaturase and the elongase enzymes, wherein a measurable difference in a level of lipid metabolites or associated cofactors in the presence of the test component compared to a control under identical conditions but in the absence of the test component is an indicator of the ability of the test component to modulate delta-5-desaturase and/or elongase enzyme activity; and (d) identifying as the compound a test component which exhibits the ability.

10 The invention further teaches a method for identifying a compound which inhibits or promotes the activity of a mammalian delta-6-desaturase enzyme and one or more enzymes selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 or ELG7, within the same host system, comprising the steps of: (a) providing a host system containing nucleic acid sequences which encode for a mammalian delta-6-desaturase and one or more mammalian  
15 elongase enzymes selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 or ELG7, operably associated with promoter regions, wherein the promoter regions are effective to initiate, terminate or regulate the level of expression of the nucleic acid sequence; (b) contacting the host system with a test component; (c) simultaneously evaluating the enzymatic activities of the delta-6-desaturase and the elongase enzymes, wherein a measurable  
20 difference in a level of lipid metabolites or associated cofactors in the presence of the test component compared to a control under identical conditions but in the absence of the test component is an indicator of the ability of the test component to modulate delta-6-desaturase and/or elongase enzyme activity; and (d) identifying as the compound a test component which exhibits the ability.

25 The invention teaches a method for identifying a compound which inhibits or promotes the activity of a mammalian delta-5- and delta-6-desaturase enzyme and/or one or more enzymes selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 or ELG7, within the same host system, comprising the steps of: (a) providing a host system containing  
30 nucleic acid sequences which encode simultaneously for a mammalian delta-5-desaturase, a mammalian delta-6-desaturase and one or more mammalian elongase enzymes selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 or ELG7, operably associated with promoter regions, wherein the promoter regions are effective to initiate, terminate or regulate the level of expression of the nucleic acid sequence; (b) contacting the  
35 host system with a test component; (c) simultaneously evaluating the enzymatic activities of the delta-5-desaturase, the delta-6-desaturase and the elongase enzymes, wherein a measurable difference in a level of lipid metabolites or associated cofactors in the presence of the test

component compared to a control under identical conditions but in the absence of the test component is an indicator of the ability of the test component to modulate delta-5- and/or delta-6-desaturase and/or elongase enzyme activity; and (d) identifying as the compound a test component which exhibits the ability.

5

The invention includes a composition for treating a PUFA disorder comprising a compound which modulates two or more human polynucleotides from control regions selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6, ELG7, delta-5-desaturase, delta-6-desaturase and a pharmaceutically acceptable carrier.

10

The invention includes a method for detecting the presence of or the predisposition for a PUFA disorder, the method comprising determining the level of expression of two or more expression products of genes selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6, ELG7, delta-5-desaturase, delta-6-desaturase, in a subject relative to a predetermined control level of expression, wherein any modified expression of the expression products as compared to the control is indicative of the presence of or the predisposition for a PUFA disorder.

15

The invention further includes an antibody immunoreactive with a polypeptide of the invention or an immunogenic portion thereof. The invention includes an antibody immunoreactive with an elongase polypeptide selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7, or an immunogenic portion thereof.

20

The invention teaches a method for screening a medium for an elongase polypeptide of the invention or selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7, comprising: (a) labelling an antibody of the invention with a marker molecule to form a conjugate; (b) exposing the conjugate to the medium; and (c) determining whether there is binding between the conjugate and a biomolecule in the medium, wherein the binding indicates the presence of the polypeptide.

25

30

The invention teaches a method for screening a medium for an elongase polypeptide of the invention or selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7, comprising: (a) exposing an antibody of the invention to the medium; (b) exposing the antibody to a marker molecule; and (c) determining whether there is binding between the marker molecule and a biomolecule in the medium, wherein the binding indicates the presence of the polypeptide.

35



The invention includes compounds identified by the method of the inventions.

5 The invention further includes a method for diagnosing the presence of or a predisposition for a PUFA disorder in a subject by detecting alterations in the elongation of PUFA in a peripheral blood leukocyte obtained from the subject. The invention includes a method for monitoring the development of a PUFA disorder in a subject by detecting alterations in the elongation of PUFA in a peripheral blood leukocyte obtained from the subjects. The invention further teaches a method for assessing the effect of test compounds on a PUFA disorder in a subject by assessing alterations in the elongation of PUFA in a peripheral blood leukocyte  
10 obtained from the subject.

The compounds of the invention may be selected from the group consisting of small organic molecules, peptides, polypeptides, antisense molecules, oligonucleotides, polynucleotides, fatty acids and derivatives thereof.  
15

The invention further teaches the use of pebulate sulfoxide for the treatment of a disorder of the invention.

20 The disorders of the invention may be selected from the group consisting of peripheral cardiovascular disease, coronary heart disease, hypertension, atopic eczema, rheumatoid arthritis, Sjögren's syndrome, gastrointestinal disorders, viral diseases and postviral fatigue, psychiatric disorders, pre-menstrual syndrome, endometriosis, cystic fibrosis, alcoholism, congenital liver disease, Alzheimer's syndrome, cancer, diabetes and diabetic complications. The disorders of the invention may be selected from the group consisting of eczema,  
25 cardiovascular disorders (including but not limited to hypertriglyceridemia, dyslipidemia, atherosclerosis, coronary artery disease, cerebrovascular disease hypertension, and peripheral vascular disease), inflammation (including but not limited to sinusitis, asthma, pancreatitis, osteoarthritis, rheumatoid arthritis and acne), Sjögren's syndrome, gastrointestinal disorders, viral diseases and postviral fatigue, body weight disorders (including but not limited to  
30 obesity, cachexia and anorexia), psychiatric disorders, cancer, cystic fibrosis, endometriosis, pre-menstrual syndrome, alcoholism, congenital liver disease, Alzheimer's syndrome, hypercholesterolemia, autoimmune disorders, atopic disorders, acute respiratory distress syndrome, articular cartilage degradation, diabetes and diabetic complications.

**BRIEF DESCRIPTION OF THE DRAWINGS**

In the following description, the invention will be explained in detail with the aid of the accompanying figures, which illustrate preferred embodiments of the present invention and in which:

Figure 1 is a schematic diagram of the n-3 fatty acid metabolic pathways;

Figure 2 is a schematic diagram of the n-6 fatty acid metabolic pathways;

Figure 3 is a schematic diagram of the n-9 and n-7 fatty acid metabolic pathways;

Figure 4 is a chart showing a multiple alignment among the 7 human elongases, highlighting the invariant residues (marked by asterisks), the histidine box (marked by a box) and the ER retention signals (marked by boxes);

Figure 5 is a graph illustrating the Transmembrane Hidden Markov Model (TMHMM) prediction for transmembrane regions for ELG7;

Figure 6 is a diagram showing a topological model of a human elongase embedded in the endoplasmic reticulum;

Figure 7 is a schematic representation of plasmid pTh1009.1 (6744 bp). The human elongase (ELG1) coding sequence is shown between restriction sites for *KpnI* and *NotI*;

Figure 8 shows the nucleotide sequence of the control region of ELG1 between position -1877 and -2865 from the translation initiation codon, ATG. This figure corresponds to SEQ. ID. NO. 1;

Figure 9 shows the nucleotide sequence of the control region of ELG2 between position -53118 and -53626 from the translation initiation codon, ATG. This figure corresponds to SEQ. ID. NO. 2;

Figure 10 shows the nucleotide sequence of the control region of ELG3 between position -37 and -1381 from the translation initiation codon, ATG. This figure corresponds to SEQ. ID. NO. 3;

Figure 11 shows the nucleotide sequence and amino acid sequence of the ELG4 gene. This figure corresponds to SEQ. ID. NOS. 4 and 5;

Figure 12 shows a 2456 bp fragment of the nucleotide sequence of the control region of ELG4.

5 This figure corresponds to SEQ. ID. NO. 6;

Figure 13 shows the nucleotide sequence of the control region of ELG5 between position -1 and -1411 from the translation initiation codon, ATG. This figure corresponds to SEQ. ID. NO. 7;

10

Figure 14 shows the nucleotide sequence and amino acid sequence of the ELG6 gene. This figure corresponds to SEQ. ID. NOS. 8 and 9;

Figure 15 shows the nucleotide sequence of the control region of ELG6 between position -1 and -1937 from the translation initiation codon, ATG. This figure corresponds to SEQ. ID. NO. 10;

15

Figure 16 shows the nucleotide sequence and amino acid sequence of the ELG7 gene. This figure corresponds to SEQ. ID. NOS. 11 and 12;

20

Figure 17 shows the nucleotide sequence of the control region of ELG7 between position -1 and -2000 from the translation initiation codon, ATG. This figure corresponds to SEQ. ID. NO. 13;

Figure 18 is a schematic representation of plasmid pTh1009.2 (6743 bp). The human elongase (ELG1) coding sequence is shown between restriction sites for *KpnI* and *NotI*;

25

Figure 19 is a schematic representation of plasmid pLh5015.1 (7927 bp). The human elongase (ELG3) coding sequence is shown between restriction sites for *BamHI* and *XbaI*;

30

Figure 20 is a schematic representation of plasmid pGh3020.1 (6168 bp). The control region for human elongase (ELG3) is shown between two *BglII* restriction sites;

Figure 21 shows an HPLC analysis of radiolabelled methyl esters of fatty acids from yeast transformed with pTh1021.1 incubated with [1-<sup>14</sup>C]18:3n-6, [1-<sup>14</sup>C]20:4n-6, [1-<sup>14</sup>C]18:3n-3 and [1-<sup>14</sup>C]20:5n-3;

35

Figure 22 shows an HPLC analysis of radiolabelled methyl esters of fatty acids from yeast transformed with pYES2/CT incubated with [1-<sup>14</sup>C]18:3n-6, [1-<sup>14</sup>C]20:4n-6, [1-<sup>14</sup>C]18:3n-3 and [1-<sup>14</sup>C]20:5n-3;

5 Figure 23 shows an HPLC analysis of radiolabelled methyl esters of fatty acids from yeast co-expressing D6D/V5-His and ELG3, incubated with [1-<sup>14</sup>C]18:2n-6 or [1-<sup>14</sup>C]18:3n-3 and with or without galactose;

10 Figure 24 shows an HPLC analysis of radiolabelled methyl esters of fatty acids from yeast co-expressing D6D/V5-His and ELG3, incubated with [1-<sup>14</sup>C]20:4n-6 or [1-<sup>14</sup>C]20:5n-3 and with or without galactose;

15 Figure 25 shows an HPLC analysis of radiolabelled methyl esters of fatty acids from yeast co-expressing D5D/V5-His and ELG3, incubated with [1-<sup>14</sup>C]20:4n-3 or [1-<sup>14</sup>C]20:3n-6 and with or without galactose;

20 Figure 26 shows an HPLC analysis of radiolabelled methyl esters of fatty acids from yeast co-expressing D5D/V5-His and ELG3, incubated with [1-<sup>14</sup>C]18:2n-6 or [1-<sup>14</sup>C]18:3n-3 and with or without galactose.

Figure 27 shows the Northern blot analyses of ELG1, ELG2, ELG3, ELG4, ELG5 and ELG7 transcripts in a variety of human tissues.

## 25 **DETAILED DESCRIPTION OF THE INVENTION**

As mentioned above, research has indicated that increased levels of LA or DGLA are the result of decreased activities of delta-6 and delta-5-desaturase enzymes. The present inventors have found evidence that both the desaturase and elongase activities are affected in a PUFA related disorder.

30 The desaturase and elongase enzyme activities in liver microsomes from streptozotocin (STZ)-induced diabetic rats was assayed at 2 and 7 weeks post-induction. Table 1 indicates the decrease in activities compared to a control, observed during the course of the experiment. An equivalent decrease in elongation activity in STZ-induced diabetic rats has been previously  
35 reported (Suneja et al., 1990, *Biochim. Biophys. Acta*, 1042: 81-85).

**Table 1**  
**Percent Decrease of the Desaturase and Elongase Activities in Liver Microsomes from STZ-**  
**Induced Diabetic Rats**

ENZYME	% Decrease	
	2 weeks	7 weeks
D6D (18:2n-6 → 18:3n-6)	28	33
Elongase (18:3n-6 → 20:3n-6)	46	43
D5D (20:3n-6 → 20:4n-6)	33	41

5

This data, when considered in view of what is known regarding the relationship between PUFAs and disease (above), indicates that elongase genes are involved in the development and regulation of lipid associated diseases such as inflammation, hypercholesterolemia, autoimmune disorders, atopic disorders, cystic fibrosis, psychiatric disorders, cancer, acute respiratory distress syndrome, articular cartilage degradation, arthritis, diabetes and diabetic complications. Since PUFAs are involved in a number of cell regulatory processes, the elongase genes and gene products represent realistic drug targets for the treatment or prevention of fatty acid associated diseases.

15 The present inventors used bioinformatic techniques to identify and analyze 7 human elongase genes (ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7). The amino acid sequences of the 7 human elongases were compared using a ClustalW algorithm (Thompson et al., 1994, *Nucl. Acids Res.*, 22: 4673-4680). One highly conserved motif, a histidine box containing 3 histidine residues, found also in a number of membrane-bound desaturases, is common to all 7 sequences. Twenty five other invariant residues, suggesting their critical importance in the catalytic activity and structure of the elongases, are identified in the multiple alignment where they are indicated by asterisk (see Figure 4).

25 Table 2 shows the percent identity among the 7 human elongases. The percent identities range from a low of 17% (ELG3/ELG5 and ELG3/ELG6) to a high of 55% (ELG1/ELG4).

**Table 2**  
**Percent Identities Among the 7 Human Elongases**

	ELG1	ELG2	ELG3	ELG4	ELG5	ELG6	ELG7
ELG1	100						
ELG2	30	100					
ELG3	29	54	100				
ELG4	55	31	34	100			
ELG5	18	18	17	22	100		
ELG6	21	18	17	22	43	100	
ELG7	33	37	37	36	18	19	100

Based on a hidden Markov model for predicting transmembrane regions (Sonnhammer et al.,  
5 1998, In *Proc. of Sixth Int. Conf. on Intelligent Systems for Molecular Biology*, AAAI Press,  
CA, pp. 175-182), this family of seven elongases has 7 membrane spanning regions (Figure 5).  
These regions are highly conserved with respect to position in the amino acid sequences of the  
7 elongases. The invariant histidine box is predicted to be embedded in the fourth  
transmembrane region. This differs from that of the membrane-bound desaturases wherein the  
10 three conserved histidine boxes are predicted to be in cytosolic loops (Shanklin et al., 1994,  
*Biochemistry*, 33: 12787-12794). The present model for the human elongases encompasses a  
ring of transmembrane domains enclosing an inner catalytic cavity for insertion of fatty acyl  
chains. A proposed topological model of the elongases embedded in the endoplasmic  
reticulum (ER) is shown in Figure 6.

15

The present inventors have discovered that each of the proteins has an ER retention signal  
(Jackson et al., 1990, *EMBO J.*, 9: 3153-3162 and Nilsson T. and Warren G., 1994, *Curr.*  
*Opin. Cell Biol.*, 6: 517-521) at the carboxyl terminus. In ER resident proteins with a type I  
topology (amino terminus in the lumen), the signal has been shown to consist of two critical  
20 lysines, which are in a -3 and a -4/-5 position relative to the carboxyl terminus in their  
cytosolic, exposed tails (K[X]KXX, where X is any amino acid). Each of the elongases has  
such a retention signal. Both ELG2 and ELG5, however, have modified forms of this signal

wherein the two critical lysines are found at positions -2 and -5, and -3 and -6, respectively (Figure 4).

### *ELG1 Gene and Polypeptide*

5  
BLASTP of the GenBank NR database with yeast ELO1 identified a protein with unknown function, CGI-88, as a potential elongase (GenBank Accession No. AAD34083). Initial cloning indicated that the cDNA sequence from which this protein was deduced (GenBank Accession No. AF151846) has a one base deletion at position 566 of the CDS. The present  
10 inventors' clone has an extra C residue at position 566 in the CDS which results in a protein, termed ELG1 by the present inventors, with a different, and longer, C-terminus than CGI-88. Since then, a gene (GenBank Accession No. AK001653) coding for a protein with no assigned function, which differs from ELG1 by one amino acid, has been submitted to GenBank (Accession No. BAA91813). The deduced amino acid sequence of ELG1 contains a F68S  
15 substitution.

The cDNA coding for ELG1 was obtained by PCR and cloned into the yeast expression vector pYES2/CT. The nucleotide sequence was verified by DNA sequencing and the resulting plasmid was designated pTh1009.1 (Figure 7).

20  
Yeast cells transformed with pTh1009.1 and expressing ELG1 were shown to convert 18:3n-6 to 20:3n-6, 20:4n-6 to 22:4n-6 and 24:4n-6, 18:3n-3 to 20:3n-3, and 20:5n-3 to 22:5n-3 (refer to Table 3 in Example 19). Yeast cells transformed with the pYES2/CT vector did not elongate any of these substrates. This proved that the ELG1 gene encodes a PUFA elongase.  
25 There is no published data demonstrating that this protein is a PUFA elongase. Mukerji et al. (PCT Application WO 00/12720) indicate that this protein, referred to as HS2, might be a PUFA elongase. They did not clone the coding sequence nor determine function.

30  
The mouse ortholog of human ELG1, Ssc1 (GenBank Accession No. AF170907), has been implicated in fatty acid elongation due to its ability to complement yeast ELO mutants. Furthermore, Ssc1 gene expression correlates with elongase activity in brains of myelin-deficient mouse mutants (Tvrdik et al., 2000, *J. Cell Biol.*, 149: 707-717). Mouse Ssc1 is 92% identical and 97% similar to human ELG1.

35  
Exons for ELG1 were mapped onto genomic DNA from human chromosome 1 (GenBank Accession No. AL139289). The gene was found to comprise 7 coding exons spanning 1.7 kb.

Using bioinformatic techniques, the control region of the ELG1 gene was identified and mapped out. By searching GenBank's EST division using BLASTN with genomic DNA and CDS for the ELG1 gene, a number of different ESTs were identified containing 5' UTR for the gene. There were 2 families of such ESTs each arising from different upstream exons which exclusively contain 5' UTR. The first exon has its 3' position at -2306 while the second exon has its 3' position at -1877 from the translation initiation codon, ATG. A 128 bp fragment of another EST (GenBank Accession No. AI373530) was also identified approximately 2.9 kb upstream of the ATG. The control region between positions -1877 and -2865 from the translation initiation codon, ATG is shown in Figure 8. A repetitive element is further identified upstream of -3600.

Northern blot studies evaluating tissue distribution showed that the ~1.3 kb ELG1 transcript is expressed in all tissues examined, with highest levels in kidney, brain, heart and placenta (Figure 27).

#### *ELG2 Gene and Polypeptide*

BLASTP of the GenBank NR database with yeast ELO1 identified a protein with unknown function (GenBank Accession No. CAB41293, since withdrawn) as a potential elongase. This protein sequence was deduced from genomic DNA (GenBank Accession No. AL034374) and represents only a partial sequence. Using GeneTrapper technology (Gibco BRL) the complete coding sequence of this protein, termed ELG2 by the present inventors, was cloned and the nucleotide sequence determined by DNA sequencing. Since then, the ELG2 coding sequence and deduced protein sequence have been submitted to GenBank (Accession Nos. AF231981 and AAF70631, respectively).

The cDNA coding for ELG2 was obtained by PCR and cloned into the yeast expression vector pYES2/CT. The sequence was verified by DNA sequencing and the resulting plasmid was designated pTh1014.1.

Yeast cells transformed with pTh1014.1 and expressing ELG2 were shown to elongate 18:3n-6 to 20:3n-6 and 22:3n-6, 20:4n-6 to 22:4n-6 and 24:4n-6, 18:3n-3 to 20:3n-3, and 20:5n-3 to 22:5n-3 (refer to Table 3 in Example 19). Yeast transformed with the pYES2/CT vector did not elongate any of these substrates. This proved that the ELG2 gene encodes a PUFA elongase. It has been reported that this gene, referred to as HELO or HSELO, encodes a protein that is involved in the elongation of a variety of PUFAs including 18:3n-6, 20:4n-6,



18:4n-3, 20:5n-3 and 18:3n-3 (Leonard et al., 2000, *Biochem. J.*, 350: 765-770 and Mukerji et al., PCT Application WO 00/12720).

Exons for ELG2 were mapped onto genomic DNA from human chromosome 6 (GenBank  
5 Accession No. AL034374). The gene was found to comprise 7 coding exons spanning 26.5 kb.

Using bioinformatic techniques, the control region of the ELG2 gene was identified and mapped out. Using sequence data from the present inventors' clones obtained by GeneTrapper technology, 5' UTR was identified in an exon approximately 53 kb upstream of the ATG. This  
10 finding was corroborated by searching GenBank's EST division using BLASTN with the ELG2 CDS. Two ESTs were identified (GenBank Accession Nos. AA282396 and BE779576) which mapped to the same upstream exon. The control region between positions -53118 and -53626 from the translation initiation codon, ATG is shown in Figure 9. Sequence from which an EST is derived (GenBank Accession No. AA557341) lies immediately upstream of this  
15 region. A repetitive element is identified approximately 1.4 kb further upstream from the 3' end of this 5' UTR-containing exon.

Northern blot studies evaluating tissue distribution showed that the ~2.8 kb ELG2 transcript is expressed in all tissues examined, with highest levels in brain, heart and kidney, and moderate  
20 levels in the liver (Figure 27).

#### *ELG3 Gene and Polypeptide*

BLASTP of the GenBank NR database with yeast ELO1 identified a protein with unknown  
25 function (GenBank Accession No. BAA91096), as a potential elongase. This protein was deduced from cDNA (GenBank Accession No. AK000341) and is termed ELG3 by the present inventors.

The cDNA coding for ELG3 was obtained by PCR and cloned into the yeast expression vector  
30 pYES2/CT. The nucleotide sequence was verified by DNA sequencing and the resulting plasmid was designated pTh1015.1. In comparison to GenBank Accession No. BAA91096, the protein encoded by the ELG3 gene contains two amino acid substitutions, T31M and V179L.

35 Yeast cells transformed with pTh1015.1 and expressing ELG3 were shown to elongate 18:3n-6 to 20:3n-6, 20:4n-6 to 22:4n-6 and 24:4n-6, 18:3n-3 to 20:3n-3, and 20:5n-3 to 22:5n-3 and

24:5n-3 (refer to Table 3 in Example 19). Yeast transformed with the pYES2/CT vector did not elongate any of these substrates. This proved that ELG3 encodes a PUFA elongase. There is no published data demonstrating that this protein is a PUFA elongase. However, Mukerji et al. (PCT Application WO 00/12720) indicate that an EST (GenBank Accession No.

- 5 AI815960), found by the present inventors to represent a portion of the CDS of ELG3, may encode a partial PUFA elongase. They did not clone the coding sequence derived from this EST nor determine its function.

- 10 The mouse ortholog of human ELG3, Ssc2 (GenBank Accession No. AF170908), has been identified as putatively involved in fatty acid elongation. However, enzymatic function has not been confirmed (Tvrdik et al., 2000, *J. Cell Biol.*, 149: 707-717). Mouse Ssc2 is 88% identical and 94% similar to human ELG3.

- 15 Exons for ELG3 were mapped onto genomic DNA from human chromosome 6 (GenBank Accession No. AL121955). The gene was found to comprise 8 coding exons spanning 60.5 kb.

- 20 Using bioinformatic techniques, the control region of the ELG3 gene was identified and mapped out. By searching GenBank using BLASTN with genomic DNA and CDS for the ELG3 gene, 2 sequences (GenBank Accession Nos. BE778035 and AK000341) were identified containing 84 bp of 5' UTR immediately upstream of the initiation codon, ATG. The control region between positions -37 and -1381 from the translation initiation codon, ATG was cloned (see Example 11) and is shown in Figure 10.

- 25 Northern blot studies evaluating tissue distribution showed that the ~4.4 kb ELG3 transcript is moderately expressed in brain, with lower levels in heart, liver and placenta (Figure 27). This transcript was not detected in any of the other tissues examined.

#### *ELG4 Gene and Polypeptide*

- 30 BLASTP of the GenBank NR database with yeast ELO1 identified a protein with unknown function (GenBank Accession No. CAB70777) as a potential elongase. This protein sequence was deduced from cDNA (GenBank Accession No. AL137506) and represents only a partial sequence. Using GeneTrapper technology (Gibco BRL) and PCR amplification the full coding sequence for this protein, termed ELG4 by the present inventors, was cloned. The cDNA  
35 sequence was determined by DNA sequencing. The coding sequence and amino sequence of ELG4 are shown in Figure 11. Since then, Kawakami and coworkers have submitted a cDNA sequence to GenBank (Accession No. AK027216) that is similar to ELG4. However, in

comparison to ELG4 it does not contain the first 31 nucleotides of the coding sequence, has several nucleotide substitutions and has a one nucleotide insertion.

5 The cDNA coding for ELG4 was obtained by PCR and cloned into the yeast expression vector pYES2/CT. The sequence was verified by DNA sequencing and the resulting plasmid was designated pTh1021.1.

10 Yeast cells transformed with pTh1021.1 and expressing ELG4 were shown to elongate 18:3n-6 to 20:3n-6 and 22:3n-6, 20:4n-6 to 22:4n-6 and 24:4n-6, and 18:3n-3 to 20:3n-3 and 22:3n-3, and 20:5n-3 to 22:5n-3 and 24:5n-3 (Refer to Table 3 in Example 19 and Figure 21). Yeast transformed with the pYES2/CT vector did not elongate any of these substrates. This proved that the ELG4 gene encodes a PUFA elongase.

15 Exons for ELG4 were mapped onto genomic DNA from human chromosome 5 (GenBank Accession No. AC021601). The gene was found to comprise 7 coding exons spanning at least 32 kb.

20 Using bioinformatic techniques, the control region of the ELG4 gene was identified and mapped out. Using sequence data from the present inventors' clones obtained by GeneTrapper technology, the 5' UTR was identified in 3 consecutive, alternatively spliced, upstream exons from the exon containing the initiation codon, ATG. The most immediate upstream exon is approximately 12 kb upstream, the next exon is over 13 kb upstream and the farthest upstream exon is at least 19 kb upstream from the ATG. The control region containing a 2456 bp fragment with its end at the 3' end of this first (most upstream) exon is shown in Figure 12. It is flanked at its 5' end by a repetitive element.

30 Northern blot studies evaluating tissue distribution showed that the ~4.3 kb ELG4 transcript is highly expressed in kidney and moderately expressed in brain and heart. Low levels of transcript were detected in skeletal muscle, colon, thymus, liver, small intestine and placenta (Figure 27). The transcript was not detected in spleen and peripheral blood leukocytes.

#### *ELG5 Gene and Polypeptide*

35 The cDNA sequence of a GenBank entry (Accession No. AK027031) encodes another potential elongase. The deduced protein sequence (GenBank Accession No. BAB15632) is termed ELG5 by the present inventors.

The cDNA coding for ELG5 was obtained by PCR and cloned into the yeast expression vector pYES2/CT. The nucleotide sequence was verified by DNA sequencing and the resulting plasmid was designated pTh1018.1.

- 5 Yeast cells transformed with pTh1018.1 and expressing ELG5 were shown to convert 18:3n-6 to 20:3n-6 and 18:3n-3 to 20:3n-3 (refer to Table 3 in Example 19). Yeast cells transformed with the pYES2/CT vector did not elongate either of these substrates. This proved that the ELG5 gene encodes a PUFA elongase. There is no published data demonstrating that this protein is a PUFA elongase. Mukerji et al. (PCT Application WO 00/12720) indicate that HS3,  
10 which is identical to ELG5, might be a PUFA elongase. The coding sequence was cloned, however, enzymatic function was not evaluated.

- Exons for ELG5 were mapped onto genomic DNA from human chromosome 4 (GenBank Accession Nos. AC004050, AC022952 and AP002080). The gene was found to comprise 4  
15 coding exons spanning at least 88 kb.

- Using bioinformatic techniques, the control region of the ELG5 gene was identified and mapped out. By searching GenBank's EST division using BLASTN with genomic DNA and CDS for the ELG5 gene, a number of different ESTs were identified containing 5' UTR for the  
20 gene. The control region between positions -1 and -1411 from the ATG is shown in Figure 13. This region is flanked at its 5' end by a repetitive element.

- Northern blot studies evaluating tissue distribution showed two transcripts for ELG5 (Figure 27). The ~3.0 kb transcript is highly expressed in liver, with moderate expression in brain,  
25 colon and kidney, and low expression in heart, thymus, small intestine, placenta and skeletal muscle. The ~7.6 kb transcript is expressed in moderate levels in the brain and low levels in colon, kidney and liver.

### 30 *ELG6 Gene and Polypeptide*

- ELG6 was identified by searching *Homo sapiens* sequences in GenBank's HTGS division with the coding sequences for ELG1, ELG2, ELG3, ELG4 and ELG5 using the TBLASTN algorithm. One sequence was identified as containing sequences similar to human elongases (GenBank Accession No. AL160011). This approach, however, failed to identify the  
35 beginning of the gene containing the translation initiation site. Therefore, further mapping and identification of ELG6 coding sequences was obtained using Cig30 (cold inducible membrane glycoprotein 30) from *Mus musculus* (GenBank Accession No. U97107), a protein found to be

similar to ELG6, as a template. The first coding exon of ELG6 containing the initiation codon, ATG, was identified in this manner.

5 The cDNA coding for ELG6 was obtained by PCR and cloned into the yeast expression vector pYES/CT. The nucleotide sequence was verified by DNA sequencing and the resulting plasmid was designated pTh1041.1. The coding sequence and amino sequence of ELG6 are shown in Figure 14.

10 Yeast cells transformed with pTh1041.1 and expressing ELG6 were shown to elongate 18:3n-6 to 20:3n-6 and 18:3n-3 to 20:3n-3 (refer to Table 3 in Example 19). Yeast cells transformed with the pYES2/CT vector did not elongate either of these substrates. This proved that the ELG6 gene encodes a PUFA elongase.

15 The mouse ortholog of human ELG6, Cig30 (GenBank Accession No. U97107), has been implicated in fatty acid elongation due to its ability to complement yeast ELO2 mutants. Furthermore, Cig30 gene expression correlates with elongase activity during brown fat recruitment in mice (Tvrđik et al., 1997, *J. Biol. Chem.*, 272: 31738-31746 and Tvrđik et al., 2000, *J. Cell Biol.*, 149: 707-717). Mouse Cig30 is 69% identical and 81% similar to human ELG6.

20 Since the inventors' discovery of ELG6 another record has been submitted to GenBank (GenBank Accession No. AF292387) containing genomic DNA and a partial CDS for the *Homo sapiens* Cig30 ortholog. Sequence annotations, however, do not indicate the presence of the first coding exon.

25 Exons for ELG6 were mapped onto genomic DNA from human chromosome 10 (GenBank Accession No. AL160011). The gene was found to comprise 4 coding exons spanning approximately 2.7 kb.

30 Using bioinformatic techniques, the control region of the ELG6 gene was identified and mapped out. The control region between positions -1 and -1937 from the ATG is shown in Figure 15.

35 The transcript for ELG6 was not detected in standard Northern blot analysis in any of the tissues examined (Figure 27).

*ELG7 Gene and Polypeptide*

ELG7 was identified by searching *Homo sapiens* sequences in GenBank's HTGS division with the coding sequences for ELG1, ELG2, ELG3, ELG4 and ELG5 using the TBLASTN  
5 algorithm. A number of sequences were identified containing exons with sequences similar to human elongases. One such sequence, 164 kb in length, (GenBank Accession No. AL132875) was found by the present inventors to contain a previously unidentified gene, termed ELG7, in 6 coding exons spanning approximately 30.5 kb of genomic DNA.

10 The cDNA coding for ELG7 was obtained by PCR and cloned into the yeast expression vector pYES2/CT. The nucleotide sequence was verified by DNA sequencing and the resulting plasmid was designated pTh1044.1. The coding sequence and amino sequence of ELG7 are shown in Figure 16.

15 Yeast cells transformed with pTh1044.1 and expressing ELG7 were shown to convert 18:3n-3 to 20:3n-3 (refer to Table 3 in Example 19). Yeast transformed with the pYES2/CT vector did not elongate this substrate. This proved that ELG7 encodes a PUFA elongase.

Using bioinformatic techniques, the control region of the ELG7 gene was identified and  
20 mapped out. By searching GenBank's EST division using BLASTN with genomic DNA for the ELG7 gene, a human EST containing 118 bp of 5' UTR for the gene was identified immediately upstream of the initiation codon, ATG (GenBank Accession No. BE878648). The control region between positions -1 and -2000 from the ATG is shown in Figure 17. A repetitive element is further identified upstream of -2700.

25 Northern blot studies evaluating tissue distribution showed that the ~3.0 kb ELG7 transcript is expressed in brain, thymus and placenta (Figure 27). This transcript was not detected in any of the other tissues examined.

30 *Subject Polynucleotides and Polypeptides*

The subject polynucleotides and polypeptides may be employed as research reagents and materials for discovery of treatments of and diagnostics for disease, particularly human  
35 disease, as further discussed herein.

### *Nucleotide Probes*

The nucleic acid molecules of the invention allow those skilled in the art to construct nucleotide probes for use in the detection of nucleotide sequences in biological materials. As described herein, a number of unique restriction sequences for restriction enzymes are incorporated in the nucleic acid molecule identified in the sequence listings of the subject polynucleotides, and these provide access to nucleotide sequences which code for polypeptides unique to the subject polynucleotides of the invention. Nucleotide sequences unique to the subject polynucleotides or isoforms thereof can also be constructed by chemical synthesis and enzymatic ligation reactions carried out by procedures known in the art.

A nucleotide probe may be labeled with a detectable marker such as a radioactive label which provides for an adequate signal and has sufficient half-life such as  $^{32}\text{P}$ ,  $^3\text{H}$ ,  $^{14}\text{C}$  or the like. Other detectable markers which may be used include antigens that are recognized by a specific labeled antibody, fluorescent compounds, enzymes, antibodies specific for a labeled antigen, and chemiluminescent compounds. An appropriate label may be selected with regard to the rate of hybridization and binding of the probe to the nucleotide to be detected and the amount of nucleotide available for hybridization. The nucleotide probes may be used to detect genes related to or analogous to the subject polynucleotides of the invention.

Accordingly, the present invention also provides a method of detecting the presence of nucleic acid molecules encoding a polypeptide related to or analogous to the subject polynucleotides in a sample comprising contacting the sample under hybridization conditions with one or more of the nucleotide probes of the invention labeled with a detectable marker, and determining the degree of hybridization between the nucleic acid molecule in the sample and the nucleotide probes.

Hybridization conditions which may be used in the method of the invention are known in the art and are described for example in Sambrook et al., 1989, *Molecular Cloning, 2nd Edition*, Cold Spring Harbor Laboratory Press, Cold Spring Harbour, NY. The hybridization product may be assayed using techniques known in the art. The nucleotide probe may be labeled with a detectable marker as described herein and the hybridization product may be assayed by detecting the detectable marker or the detectable change produced by the detectable marker.

### *Primers*

The identification of the nucleic acid molecule of the invention also permits the identification

and isolation, or synthesis of nucleotide sequences which may be used as primers to amplify a polynucleotide molecule of the invention, for example in polymerase chain reaction (PCR). The length and bases of the primers for use in the PCR are selected so that they will hybridize to different strands of the desired sequence and at relative positions along the sequence such  
5 that an extension product synthesized from one primer when it is separated from its template can serve as a template for extension of the other primer into a nucleic acid of defined length.

Primers which may be used in the invention are oligonucleotides i.e. molecules containing two or more deoxyribonucleotides of the nucleic acid molecule of the invention which occur  
10 naturally as in a purified restriction endonuclease digest or are produced synthetically using techniques known in the art such as, for example, phosphotriester and phosphodiester methods or automated techniques (Connolly B. A., 1987, *Nucl. Acid Res.*, 15: 3131-3139). The primers are capable of acting as a point of initiation of synthesis when placed under conditions which permit the synthesis of a primer extension product which is complementary to the DNA  
15 sequence of the invention e.g. in the presence of nucleotide substrates, an agent for polymerization such as DNA polymerase and at suitable temperature and pH. Preferably, the primers are sequences that do not form secondary structures by base pairing with other copies of the primer or sequences that form a hair pin configuration. The primer may be single or double-stranded. When the primer is double-stranded it may be treated to separate its strands  
20 before using it to prepare amplification products. The primer preferably contains between about 7 and 25 nucleotides.

The primers may be labeled with detectable markers which allow for detection of the amplified products. Suitable detectable markers are radioactive markers such as  $^{32}\text{P}$ ,  $^{35}\text{S}$ ,  $^{125}\text{I}$   
25 and  $^3\text{H}$ , luminescent markers such as chemiluminescent markers, preferably luminol and fluorescent markers, preferably dansyl chloride, fluorescein-5-isothiocyanate and 4-fluor-7-nitrobenz-2-oxa-1,3 diazole and cofactors such as biotin. It will be appreciated that the primers may contain non-complementary sequences provided that a sufficient amount of the primer contains a sequence which is complementary to a nucleic acid molecule of the invention or  
30 oligonucleotide sequence thereof, which is to be amplified. Restriction site linkers may also be incorporated into the primers allowing for digestion of the amplified products with the appropriate restriction enzymes facilitating cloning and sequencing of the amplified product.

#### *Assays - Amplifying Sequences*

35

Thus, a method of determining the presence of a nucleic acid molecule having a sequence encoding the subject polynucleotides or a predetermined oligonucleotide fragment thereof in a



sample, is provided comprising treating the sample with primers which are capable of amplifying the nucleic acid molecule or the predetermined oligonucleotide fragment thereof in a polymerase chain reaction to form amplified sequences, under conditions which permit the formation of amplified sequences and, assaying for amplified sequences.

5

The polymerase chain reaction refers to a process for amplifying a target nucleic acid sequence as generally described in Innis M.A. and Gelfand D.H., 1989, PCR Protocols, A Guide to Methods and Applications, Innis M.A., Gelfand D.H., Shinsky J.J. and White T.J. (eds), Academic Press, NY, pp. 3-12, which are incorporated herein by reference. Conditions for

10 amplifying a nucleic acid template are described in Innis M.A. and Gelfand D.H., 1989, PCR Protocols, A Guide to Methods and Applications, Innis M.A., Gelfand D.H., Shinsky J.J. and White T.J. (eds), Academic Press, NY, pp. 3-12, which is also incorporated herein by reference.

15 The amplified products can be isolated and distinguished based on their respective sizes using techniques known in the art. For example, after amplification, the DNA sample can be separated on an agarose gel and visualized, after staining with ethidium bromide, under ultraviolet (UV) light. DNA may be amplified to a desired level and a further extension reaction may be performed to incorporate nucleotide derivatives having detectable markers

20 such as radioactive labeled or biotin labeled nucleoside triphosphates. The primers may also be labeled with detectable markers. The detectable markers may be analyzed by restriction and electrophoretic separation or other techniques known in the art.

The conditions which may be employed in the methods of the invention using PCR are those

25 which permit hybridization and amplification reactions to proceed in the presence of DNA in a sample and appropriate complementary hybridization primers. Conditions suitable for the polymerase chain reaction are generally known in the art. For example, see Innis M.A. and Gelfand D.H., 1989, PCR Protocols, A Guide to Methods and Applications, Innis M.A., Gelfand D.H., Shinsky J.J. and White T.J. (eds), Academic Press, NY, pp. 3-12, which is

30 incorporated herein by reference. Preferably, the PCR utilizes polymerase obtained from thermophilic bacterium *Thermus aquaticus* (Taq polymerase, GeneAmp Kit, Perkin Elmer Cetus) or other thermostable polymerase may be used to amplify DNA template strands.

It will be appreciated that other techniques such as the Ligase Chain Reaction (LCR) and

35 Nucleic-Acid Sequence Based Amplification (NASBA) may be used to amplify a nucleic acid molecule of the invention. In LCR, two primers which hybridize adjacent to each other on the target strand are ligated in the presence of the target strand to produce a complementary strand

(Backman, 1991 and European Published Application No. 0320308, published Jun. 14, 1989). NASBA is a continuous amplification method using two primers, one incorporating a promoter sequence recognized by an RNA polymerase and the second derived from the complementary sequence of the target sequence to the first primer (U.S. Pat. No. 5,130,238 to Malek).

### *Vectors*

The present invention also teaches vectors which comprise a polynucleotide or polynucleotides of the present invention, host cells which are genetically engineered with vectors of the invention and the production of polynucleotides of the invention by recombinant techniques.

In accordance with this aspect of the invention the vector may be, for example, a plasmid vector, a single or double-stranded phage vector, or a single or double-stranded RNA or DNA viral vector. In certain embodiments in this regard, the vectors provide for specific expression. Such specific expression may be inducible expression or expression only in certain types of cells or both inducible and cell-specific. Particular among inducible vectors are vectors that can be induced for expression by environmental factors that are easy to manipulate, such as temperature and nutrient additives. A variety of vectors suitable to this aspect of the invention, including constitutive and inducible expression vectors for use in prokaryotic and eukaryotic hosts, are well known and employed routinely by those of skill in the art. Such vectors include, among others, chromosomal, episomal and virus-derived vectors, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. All of these may be used for expression in accordance with this aspect of the present invention.

The following vectors, which are commercially available, are provided by way of example. Among vectors for use in bacteria are pQE-9, pQE-16, pQE-30, pQE-40, pQE-50 and pQE-60 (Qiagen); pCRII, pCRII-TOPO, pTrcHis and pBAD-TOPO (Invitrogen); pGEM-3Z, pGEMEX-1, pET-5 (Promega); pBS phagemid vectors, Phagescript vectors, Bluescript vectors, pCAL, pET-3 and pSPUTK (Stratagene); pTrc99A, pKK223-3, pKK232-8 and pRIT2T (Pharmacia); pMAL (New England Biolabs); and pBR322 (ATCC 37017). Among eukaryotic vectors are pGAPZ, pYES2, pYES2/CT and pcDNA3.1 (Invitrogen); pCAT3 and

pGL3 (Promega); pCMV-Script, pXT1, pDual, pCMVLacI, pESC, HybriZAP2.1, ImmunoZAP and pRS (Stratagene); and pSVK3, pSVL and pMSG (Pharmacia). These vectors are listed solely by way of illustration of the many commercially available and well known vectors that are available to those of skill in the art for use in accordance with this aspect of the present invention. It will be appreciated that any other plasmid or vector suitable for, for example, introduction, maintenance, propagation or expression of a polynucleotide or polypeptide of the invention in a host may be used in this aspect of the invention. Generally, any vector suitable to maintain, propagate or express polynucleotides to express a polypeptide or polynucleotide in a host may be used for expression in this regard.

The DNA sequence in the expression vector is operatively linked to appropriate expression control sequence(s), including, for instance, a promoter to direct mRNA transcription. Promoter regions can be selected from any desired gene using vectors that contain a reporter transcription unit lacking a promoter region, such as a chloramphenicol acetyl transferase (CAT) transcription unit, downstream of restriction site or sites for introducing a candidate promoter fragment; i.e., a fragment that may contain a promoter. As is well known, introduction into the vector of a promoter-containing fragment at the restriction site upstream of the CAT gene engenders production of CAT activity, which can be detected by standard CAT assays. Vectors suitable to this end are well known and readily available, such as pKK232-8 and pCAT3. Promoters for expression of polynucleotides of the present invention include not only well known and readily available promoters, but also promoters that readily may be obtained by the foregoing technique, using a reporter gene. Among known prokaryotic promoters suitable for expression of polynucleotides and polypeptides in accordance with the present invention are the *E. coli lacI* and *lacZ* promoters, the T3 and T7 promoters, the *gpt* promoter, the lambda PR and PL promoters, and the *trp* promoter. Among known eukaryotic promoters suitable in this regard are the CMV immediate early promoter, the HSV thymidine kinase promoter, the early and late SV40 promoters, the promoters of retroviral LTRs, such as those of the Rous sarcoma virus (RSV), and metallothionein promoters, such as the mouse metallothionein-I promoter.

Vectors for propagation and expression generally will include selectable markers and amplification regions, such as, for example, those set forth in Sambrook et al., *supra*.

### *Host Cells*

As hereinbefore mentioned, the present invention also teaches host cells which are genetically engineered with vectors of the invention.

Polynucleotide constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. The subject polynucleotides or polypeptides products or isoforms or parts thereof, may be obtained by expression in a suitable host cell using techniques known in the art. Suitable host cells include prokaryotic or eukaryotic organisms or cell lines, for example bacterial, mammalian, yeast, or other fungi, viral, plant or insect cells. Methods for transforming or transfecting cells to express foreign DNA are well known in the art (See for example, Itakura et al., U.S. Pat. No. 4,704,362; Murray et al., U.S. Pat. No. 4,801,542; McKnight et al., U.S. Pat. No. 4,935,349; Hagen et al., U.S. Pat. No. 4,784,950; Axel et al., U.S. Pat. No. 4,399,216; Goeddal et al., U.S. Pat. No. 4,766,075 and Sambrook et al., 1989, *Molecular Cloning, 2nd Edition*, Cold Spring Harbor Laboratory Press, Cold Spring Harbour, NY all of which are incorporated herein by reference). Representative examples of appropriate hosts include bacterial cells, such as Streptococci, Staphylococci, *E. coli*, Streptomyces and *Bacillus subtilis*; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS-1, ZR-75-1, Chang, HeLa, C127, 3T3, HepG2, BHK, 293 and Bowes melanoma cells; and plant cells.

Host cells can be genetically engineered to incorporate polynucleotides and express polynucleotides of the present invention. Introduction of polynucleotides into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, transfection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., 1986, *Basic Methods in Molecular Biology*, Elsevier, NY and Sambrook et al., 1989, *Molecular Cloning, 2nd Edition*, Cold Spring Harbor Laboratory Press, Cold Spring Harbour, NY.

### *Production of the Subject Polypeptides*

As hereinbefore mentioned, the present invention also teaches the production of polynucleotides of the invention by recombinant techniques.

The subject polynucleotides encode polypeptides which are the mature protein plus additional amino- or carboxyl-terminal amino acids, or amino acids interior to the mature polypeptide (when the mature form has more than one polypeptide chain, for instance). Such sequences may play a role in processing of a protein from precursor to a mature form, may allow protein transport, may lengthen or shorten protein half-life or may facilitate manipulation of a protein for assay or production, among other things. Generally, as is the case *in vivo*, the additional amino acids may be processed away from the mature protein by cellular enzymes.

A precursor protein, having the mature form of the polypeptide fused to one or more prosequences may be an inactive form of the polypeptide. When prosequences are removed such inactive precursors generally are activated. Some or all of the prosequences may be removed before activation. Generally, such precursors are called proproteins.

Thus, a polynucleotide of the present invention may encode a mature protein, a mature protein plus a leader sequence (which may be referred to as a preprotein), a precursor of a mature protein having one or more prosequences which are not the leader sequences of a preprotein, or a preproprotein, which is a precursor to a proprotein, having a leader sequence and one or more prosequences, which generally are removed during processing steps that produce active and mature forms of the polypeptide.

The polypeptides of the invention may be prepared by culturing the host/vector systems described above, in order to express the recombinant polypeptides. Recombinantly produced subject protein or parts thereof, may be further purified using techniques known in the art such as commercially available protein concentration systems, by salting out the protein followed by dialysis, by affinity chromatography, or using anion or cation exchange resins.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using DNA derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook et al., *supra*.

Polynucleotides of the invention, encoding the heterologous structural sequence of a polynucleotide or polypeptide of the invention generally will be inserted into a vector using standard techniques so that it is operably linked to the promoter for expression. The polynucleotide will be positioned so that the transcription start site is located appropriately 5' to a ribosome binding site. The ribosome binding site will be 5' to the AUG that initiates

translation of the polynucleotide or polypeptide to be expressed. Generally, there will be no other open reading frames that begin with an initiation codon, usually AUG, and lie between the ribosome binding site and the initiation codon. Also, generally, there will be a translation stop codon at the end of the expressed polynucleotide and there will be a polyadenylation  
5 signal in constructs for use in eukaryotic hosts. Transcription termination signal appropriately disposed at the 3' end of the transcribed region may also be included in the polynucleotide construct.

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the  
10 periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the expressed polynucleotide or polypeptide. These signals may be endogenous to the polynucleotide or they may be heterologous signals. Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, or other  
15 such methods known to those skilled in the art. A subject polynucleotide or polypeptide can be recovered and purified from recombinant cell cultures by known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most  
20 preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding protein may be employed to regenerate active conformation when the polynucleotide is denatured during isolation and or purification.

A nucleic acid molecule of the invention may be cloned into a glutathione S-transferase (GST)  
25 gene fusion system for example the pGEX-1T, pGEX-2T and pGEX-3X of Pharmacia. The fused gene may contain a strong *lac* promoter, inducible to a high level of expression by IPTG, as a regulatory element. Thrombin or factor Xa cleavage sites may be present which allow proteolytic cleavage of the desired polypeptide from the fusion product. The glutathione S-transferase-subject polypeptide fusion protein may be easily purified using a glutathione  
30 sepharose 4B column, for example from Pharmacia. The 26 kDa glutathione S-transferase polypeptide can be cleaved by thrombin (pGEX-1T or pGEX-2T) or factor Xa (pGEX-3X) and resolved from the polypeptide using the same affinity column. Additional chromatographic steps can be included if necessary, for example Sephadex or DEAE cellulose. The two enzymes may be monitored by protein and enzymatic assays and purity may be  
35 confirmed using SDS-PAGE.

The subject protein or parts thereof may also be prepared by chemical synthesis using techniques well known in the chemistry of proteins such as solid phase synthesis (Merrifield, 1964, *J. Am. Chem. Assoc.*, 85: 2149-2154) or synthesis in homogenous solution (Houbenweyl et al., 1987, *Methods of Organic Chemistry*, Wansch E. (ed), Vol. 15 I and II, Thieme, Germany).

Within the context of the present invention, the subject polypeptide includes various structural forms of the primary protein which retain biological activity. For example, the subject polypeptide may be in the form of acidic or basic salts or in neutral form. In addition, individual amino acid residues may be modified by oxidation or reduction. Furthermore, various substitutions, deletions or additions may be made to the amino acid or nucleic acid sequences, the net effect being that biological activity of the subject polypeptide is retained. Due to code degeneracy, for example, there may be considerable variation in nucleotide sequences encoding the same amino acid.

The polypeptide may be expressed in a modified form, such as a fusion protein, and may include not only secretion signals but also additional heterologous functional regions. Thus, for instance, a region of additional amino acids, particularly charged amino acids, may be added to the carboxyl- or amino-terminus of the polypeptide to improve stability and persistence in the host cell during purification or during subsequent handling and storage. Also, fusion proteins may be added to the polynucleotide or polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polynucleotide or polypeptide. The addition of peptide moieties to polynucleotides or polypeptides to engender secretion or excretion, to improve stability or to facilitate purification, among others, are familiar and routine techniques in the art. In drug discovery, for example, proteins have been fused with antibody Fc portions for the purpose of high-throughput screening assays to identify antagonists (Bennett et al., 1995, *J. Mol. Recognit.*, 8: 52-58, and Johanson et al., 1995, *J. Biol. Chem.*, 270: 9459-9471).

30

### *Antibodies*

With respect to protein-based testing, antibodies can be generated to the elongase gene product using standard immunological techniques, fusion proteins or synthetic peptides as described herein. Monoclonal antibodies can also be produced using now conventional techniques such as those described in Waldmann T.A., 1991, *Science*, 252: 1657-1662 and Harlow E. and Lane D. (eds.), 1988, *Antibodies: A Laboratory Manual*, Cold Harbour Press, Cold Harbour, NY. It

will also be appreciated that antibody fragments, i.e. Fab' fragments, can be similarly employed. Immunoassays, for example ELISAs, in which the test sample is contacted with antibody and binding to the gene product detected, can provide a quick and efficient method of determining the presence and quantity of the elongase gene product. For example, the  
5 antibodies can be used to test the effect of pharmaceuticals in subjects enrolled in clinical trials.

Thus, the present invention also provides polyclonal and/or monoclonal antibodies and fragments thereof, and immunologic binding equivalents thereof, which are capable of  
10 specifically binding to the subject polypeptides and fragments thereof or to polynucleotide sequences from the subject polynucleotide region, particularly from the subject polypeptide locus or a portion thereof. The term "antibody" is used both to refer to a homogeneous molecular entity, or a mixture such as a serum product made up of a plurality of different molecular entities. Polypeptides may be prepared synthetically in a peptide synthesizer and  
15 coupled to a carrier molecule (e.g., keyhole limpet hemocyanin) and injected over several months into rabbits. Rabbit sera is tested for immunoreactivity to the subject polypeptide or fragment. Monoclonal antibodies may be made by injecting mice with the protein polypeptides, fusion proteins or fragments thereof. Monoclonal antibodies are screened by ELISA and tested for specific immunoreactivity with subject polypeptide or fragments thereof  
20 (Harlow E. and Lane D. (eds.), 1988, *Antibodies: A Laboratory Manual*, Cold Harbour Press, Cold Harbour, NY). These antibodies are useful in assays as well as pharmaceuticals.

Once a sufficient quantity of desired polypeptide has been obtained, it may be used for various purposes. A typical use is the production of antibodies specific for binding. These antibodies  
25 may be either polyclonal or monoclonal, and may be produced by *in vitro* or *in vivo* techniques well known in the art. For production of polyclonal antibodies, an appropriate target immune system, typically mouse or rabbit, is selected. Substantially purified antigen is presented to the immune system in a fashion determined by methods appropriate for the animal and by other parameters well known to immunologists. Typical routes for injection are in footpads,  
30 intramuscularly, intraperitoneally, or intradermally. Of course, other species may be substituted for mouse or rabbit. Polyclonal antibodies are then purified using techniques known in the art, adjusted for the desired specificity.

An immunological response is usually assayed with an immunoassay. Normally, such  
35 immunoassays involve some purification of a source of antigen, for example, that produced by the same cells and in the same fashion as the antigen. A variety of immunoassay methods are well known in the art, such as in Harlow E. and Lane D. (eds.), 1988, *Antibodies: A*



*Laboratory Manual*, Cold Harbour Press, Cold Harbour, NY, or Goding J.W., 1996, *Monoclonal Antibodies: Principles and Practice: Production and Application of Monoclonal Antibodies in Cell Biology, Biochemistry and Immunology*, 3<sup>rd</sup> edition, Academic Press, NY.

- 5 Monoclonal antibodies with affinities of  $10^8 \text{ M}^{-1}$  or preferably  $10^9$  to  $10^{10} \text{ M}^{-1}$  or stronger will typically be made by standard procedures as described in Harlow E. and Lane D. (eds.), 1988, *Antibodies: A Laboratory Manual*, Cold Harbour Press, Cold Harbour, NY or Goding J.W., 1996, *Monoclonal Antibodies: Principles and Practice: Production and Application of Monoclonal Antibodies in Cell Biology, Biochemistry and Immunology*, 3<sup>rd</sup> edition, Academic Press, NY. Briefly, appropriate animals will be selected and the desired immunization protocol followed. After the appropriate period of time, the spleens of such animals are excised and individual spleen cells fused, typically, to immortalized myeloma cells under appropriate selection conditions. Thereafter, the cells are clonally separated and the supernatants of each clone tested for their production of an appropriate antibody specific for
- 10
- 15 the desired region of the antigen.

- Other suitable techniques involve *in vitro* exposure of lymphocytes to the antigenic polypeptides, or alternatively, to selection of libraries of antibodies in phage or similar vectors (Huse et al., 1989, *Science*, 246: 1275-1281). The polypeptides and antibodies of the present invention may be used with or without modification. Frequently, polypeptides and antibodies will be labeled by joining, either covalently or non-covalently, a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include
- 20 radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent agents, chemiluminescent agents, magnetic particles and the like. Patents teaching the use of such labels include U.S. Pat. Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149 and 4,366,241. Also, recombinant immunoglobulins may be produced (see U.S. Pat. No. 4,816,567).
- 25

#### *Generation of Polyclonal Antibody Against the Subject Polynucleotide*

- 30 Sequences of the subject polynucleotide coding sequence are expressed as fusion protein in *E. coli*. The overexpressed protein is purified by gel elution and used to immunize rabbits and mice using a procedure similar to the one described by Harlow E. and Lane D. (eds.), 1988, *Antibodies: A Laboratory Manual*, Cold Harbour Press, Cold Harbour, NY. This procedure has been shown to generate antibodies against various other proteins (for example, see Kraemer et al., 1993, *J. Lipid Res.*, 34: 663-671).
- 35

Briefly, a stretch of coding sequence selected from the subject polynucleotide is cloned as a fusion protein in plasmid pET5A (Novagen, WI) or pMAL system (New England Biolabs, U.S.). After induction with IPTG, the overexpression of a fusion protein with the expected molecular weight is verified by SDS-PAGE. Fusion protein is purified from the gel by electroelution. The identification of the protein as the subject polypeptide fusion product can be verified by protein sequencing at the N-terminus. Next, the purified protein is used as immunogen in rabbits. Rabbits are immunized with 100 µg of the protein in complete Freund's adjuvant and boosted twice in 3 week intervals, first with 100 µg of immunogen in incomplete Freund's adjuvant followed by 100 µg of immunogen in PBS. Antibody containing serum is collected two weeks thereafter.

This procedure is repeated to generate antibodies against the mutant forms of the subject polypeptide. These antibodies, in conjunction with antibodies to wild type subject polypeptide, are used to detect the presence and the relative level of the mutant forms in various tissues and biological fluids.

#### *Generation of Monoclonal Antibodies Specific for the Subject Polypeptide*

Monoclonal antibodies are generated according to the following protocol. Mice are immunized with immunogen comprising intact subject polypeptide or its peptides (wild type or mutant) conjugated to keyhole limpet hemocyanin using glutaraldehyde or EDC as is well known.

The immunogen is mixed with an adjuvant. Each mouse receives four injections of 10 to 100 µg of immunogen and after the fourth injection blood samples are taken from the mice to determine if the serum contains antibody to the immunogen. Serum titer is determined by ELISA or RIA. Mice with sera indicating the presence of antibody to the immunogen are selected for hybridoma production.

Spleens are removed from immune mice and a single cell suspension is prepared as described by Harlow E. and Lane D. (eds.), 1988, *Antibodies: A Laboratory Manual*, Cold Harbour Press, Cold Harbour, NY. Cell fusions are performed essentially as described by Kohler G. and Milstein C., 1975, *Nature*, 256: 495-497. Briefly, P3.65.3 myeloma cells (American Type Culture Collection, Rockville, MD) are fused with immune spleen cells using polyethylene glycol as described by Harlow E. and Lane D. (eds.), 1988, *Antibodies: A Laboratory Manual*, Cold Harbour Press, Cold Harbour, NY. Cells are plated at a density of  $2 \times 10^5$  cells/well in 96

well tissue culture plates. Individual wells are examined for growth and the supernatants of wells with growth are tested for the presence of subject polypeptide specific antibodies by ELISA or RIA using wild type or mutant target protein. Cells in positive wells are expanded and subcloned to establish and confirm monoclonality.

5

Clones with the desired specificities are expanded and grown as ascites in mice or in a hollow fiber system to produce sufficient quantities of antibody for characterization and assay development.

10

*Sandwich Assay for the Subject Polypeptide*

Monoclonal antibody is attached to a solid surface such as a plate, tube, bead, or particle. Preferably, the antibody is attached to the well surface of a 96-well ELISA plate. A 100  $\mu$ l sample (e.g., serum, urine, tissue cytosol) containing the subject polypeptide/protein (wild-type or mutant) is added to the solid phase antibody. The sample is incubated for 2 hrs at room temperature. Next the sample fluid is decanted, and the solid phase is washed with buffer to remove unbound material. One hundred  $\mu$ l of a second monoclonal antibody (to a different determinant on the subject polypeptide/protein) is added to the solid phase. This antibody is labeled with a detector molecule or atom (e.g.,  $^{125}$ I, enzyme, fluorophore, or a chromophore) and the solid phase with the second antibody is incubated for two hrs at room temperature. The second antibody is decanted and the solid phase is washed with buffer to remove unbound material.

The amount of bound label, which is proportional to the amount of subject polypeptide/protein present in the sample, is quantitated. Separate assays are performed using monoclonal antibodies which are specific for the wild-type subject polypeptide as well as monoclonal antibodies specific for each of the mutations identified in subject polypeptide.

*Detecting Presence of or Predisposition for Disorders Affected by Lipid Metabolism and Monitoring Treatment of Same*

As previously discussed, lipid metabolism is frequently dysregulated in disease. It is likely that genetic polymorphisms in elongase genes will contribute to disease susceptibility.

35

The subject polynucleotides taught herein are useful to detect genetic polymorphisms of the

subject polynucleotides, or to detecting changes in the level of expression of the subject polynucleotides, as a diagnostic tool. Detection of an aberrant form of the subject polynucleotide, or a decrease or increase in the level of expression of the subject polynucleotide in a eukaryote, particularly a mammal, and especially a human, will provide a method for diagnosis of a disease. Eukaryotes (herein also "individual(s)"), particularly mammals, and especially humans, exhibiting genetic polymorphisms of the subject polynucleotides, or changes in expression of the subject polynucleotides may be detected by a variety of techniques.

Since elongase genes are widely expressed, test samples of the subject can be obtained from a variety of tissues including blood. An elongase gene test can also be included in panels of prenatal tests since elongase genes, DNA, RNA or protein can also be assessed in amniotic fluid. Quantitative testing for elongase gene transcript and gene product is thus also contemplated within the scope of the present invention.

Nucleic acid and protein-based methods for screening genetic polymorphisms in elongase genes are all within the scope of the present teachings. For example, knowing the sequence of the elongase gene, DNA or RNA probes can be constructed and used to detect mutations in elongase genes through hybridization with genomic DNA in a tissue such as blood using conventional techniques. RNA or cDNA probes can be similarly probed to screen for mutations in elongase genes or for quantitative changes in expression. A mixture of different probes, i.e. "probe cocktail", can also be employed to test for more than one mutation.

With respect to nucleic acid-based testing, genomic DNA may be used directly for detection of a specific sequence or may be amplified enzymatically *in vitro* by using PCR prior to analysis (Saiki et al., 1985, *Science*, 230: 1350-1353 and Saiki et al., 1986, *Nature*, 324: 163-166). Reviews of this subject have been presented by Caskey C.T., 1989, *Science*, 236: 1223-1229 and by Landegren et al., 1989, *Science*, 242: 229-237. The detection of specific DNA sequence may be achieved by methods such as hybridization using specific oligonucleotides (Wallace et al., 1986, *Cold Spring Harbour Symp. Quant. Biol.*, 51: 257-261), direct DNA sequencing (Church et al., 1988, *Proc. Natl. Acad. Sci.*, 81: 1991-1995, the use of restriction enzymes (Flavell et al., 1978, *Cell*, 15: 25-41; Geever et al., 1981, *Proc. Natl. Acad. Sci.*, 78: 5081-5085), discrimination on the basis of electrophoretic mobility in gels with denaturing reagent (Myers et al., 1986, *Cold Spring Harbour Sym. Quant. Biol.*, 51: 275-284), RNase protection (Myers et al., 1985, *Science*, 230: 1242-1246), chemical cleavage (Cotton et al., 1985, *Proc. Natl. Acad. Sci.*, 85: 4397-4401), and the ligase-mediated detection procedure (Landegren et al., 1988, *Science*, 241: 1077-1080). Using PCR, characterization of the level of

or condition of the subject polynucleotides present in the individual may be made by comparative analysis.

5 With respect to protein-based testing, antibodies can be generated to the elongase gene product using standard immunological techniques, fusion proteins or synthetic peptides as described herein.

10 With the characterization of the elongase gene product and its function, functional assays can also be used for elongase gene diagnosis and screening and to monitor treatment. For example, enzymatic testing to determine levels of gene function, rather than direct screening of the elongase gene or product, can be employed. Testing of this nature has been utilized in other diseases and conditions, such as in Tay-Sachs.

15 The invention thus provides a process for detecting disease by using methods known in the art and methods described herein to detect changes in expression of or mutations to the subject polynucleotides. For example, decreased expression of a subject polynucleotide can be measured using any one of the methods well known in the art for the quantification of polynucleotides, such as, for example, PCR, RT-PCR, DNase protection, Northern blotting and other hybridization methods. Thus, the present invention provides a method for detecting disorders affected by lipid metabolism, and a method for detecting a genetic pre-disposition for such diseases including eczema, cardiovascular disorders (including but not limited to hypertriglyceridemia, dyslipidemia, atherosclerosis, coronary artery disease, cerebrovascular disease and peripheral vascular disease), inflammation (including but not limited to sinusitis, asthma, pancreatitis, osteoarthritis, rheumatoid arthritis and acne), body weight disorders 20 (including but not limited to obesity, cachexia and anorexia), psychiatric disorders, cancer, cystic fibrosis, pre-menstrual syndrome, diabetes and diabetic complications.

#### *Drug Screening Assays*

30 The present teachings provide methods for screening compounds to identify those which enhance (agonist) or block (antagonist) the action of subject polypeptides or polynucleotides, such as its interaction with fatty acid binding molecules. The identification of the subject polynucleotides in inherited fatty acid disorders, combined with advances in the field of transgenic methods, provides the information necessary to further study human diseases. This is extraordinarily useful in modeling familial forms of fatty acid disorders and other diseases 35 of fatty acid metabolism including eczema, cardiovascular disorders (including but not limited to hypertriglyceridemia, dyslipidemia, atherosclerosis, coronary artery disease,

cerebrovascular disease and peripheral vascular disease), inflammation (including but not limited to sinusitis, asthma, pancreatitis, osteoarthritis, rheumatoid arthritis and acne), body weight disorders (including but not limited to obesity, cachexia and anorexia), psychiatric disorders, cancer, cystic fibrosis, pre-menstrual syndrome, diabetes and diabetic complications. Drug screening assays are made effective by use of the control regions of the genes described in the present invention or part of it, in a yeast based DNA-protein interaction assay (yeast one-hybrid). The use of the genes described here, or parts thereof, or the transcribed RNA in a yeast protein-protein interaction (2-hybrid) or protein-RNA interaction assays for drug screening also provide effective drug screening methods. Such interacting molecules can also be reconstructed *in vitro* for drug screening purposes.

For example, to screen for agonists or antagonists, a synthetic reaction mix, a cellular compartment, such as a membrane, cell envelope or cell wall, or a preparation of any thereof, may be prepared from a cell that expresses a molecule that binds a subject polynucleotide. The preparation is incubated with labeled polynucleotide in the absence or the presence of a candidate molecule which may be an agonist or antagonist. The ability of the candidate molecule to bind the binding molecule is reflected in decreased binding of the labeled ligand.

Effects of potential agonists and antagonists may be measured, for instance, by determining activity of a reporter system following interaction of the candidate molecule with a cell or appropriate cell preparation, and comparing the effect to a baseline (control) measurement. Reporter systems that may be useful in this regard include, but are not limited to, colorimetric labeled substrate converted into product, a reporter gene that is responsive to changes in elongase enzyme activity, and binding assays known in the art.

Another example of an assay for antagonists is a competitive assay that combines a subject polypeptide and a potential antagonist with membrane-bound subject polypeptide-binding molecules, recombinant subject polypeptide binding molecules, natural substrates or ligands, or substrate or ligand mimetics, under appropriate conditions for a competitive inhibition assay. A subject polypeptide can be labeled, such as by radioactivity or a colorimetric compound, such that the number of subject polypeptide molecules bound to a binding molecule or converted to product can be determined accurately to assess the effectiveness of the potential antagonist.

Potential antagonists include small organic molecules, peptides, polypeptides and antibodies that bind to a polynucleotide or polypeptide of the invention and thereby inhibit or extinguish its activity. Potential antagonists also may be small organic molecules, peptides, polypeptides,

such as closely related proteins or antibodies that bind the same sites on a binding molecule, without inducing subject polypeptide-induced activities, thereby preventing the action of the subject polypeptide by excluding the subject polypeptide from binding. Potential antagonists include antisense molecules (Okano et al., 1988, *EMBO J.*, 7: 3407-3412). Potential  
5 antagonists include compounds related to and derivatives of the subject polypeptides.

Potential antagonists include small organic molecules, peptides, polypeptides and antibodies that bind to a polynucleotide or polypeptide of the invention and thereby inhibit or extinguish its activity. Potential agonists may be selected from the group consisting of small organic  
10 molecules, peptides, polypeptides, antisense molecules, oligonucleotides, polynucleotides, fatty acids, and chemical and functional derivatives thereof.

Developing modulators of the biological activities of specific elongases requires differentiating elongase isozymes present in a particular assay preparation. The classical  
15 enzymological approach of isolating elongases from natural tissue sources and studying each new isozyme may be used. Another approach has been to identify assay conditions which might favor the contribution of one isozyme and minimize the contribution of others in a preparation. Still another approach is the separation of elongases by immunological means. Each of the foregoing approaches for differentiating elongase isozymes is time consuming. As  
20 a result many attempts to develop selective elongase modulators have been performed with preparations containing more than one isozyme. Moreover, elongase preparations from natural tissue sources are susceptible to limited proteolysis and may contain mixtures of active proteolytic products that have different kinetic, regulatory and physiological properties than the full length elongases.

Recombinant subject polypeptide products of the invention greatly facilitate the development of new and specific modulators. The need for purification of an isozyme can be avoided by expressing it recombinantly in a host cell that lacks endogenous elongase activity. Once a compound that modulates the activity of the elongase is discovered, its selectivity can be  
30 evaluated by comparing its activity on the particular subject enzyme to its activity on other elongase isozymes. Thus, the combination of the recombinant subject polypeptide products of the invention with other recombinant elongase products in a series of independent assays provides a system for developing selective modulators of particular elongases. Selective modulators may include, for example, antibodies and other proteins or peptides which  
35 specifically bind to the subject polypeptide or polynucleotide, oligonucleotides which specifically bind to the subject polypeptide (see Patent Cooperation Treaty International Publication No. WO 93/05182 which describes methods for selecting oligonucleotides which

selectively bind to target biomolecules) or the subject polynucleotide (e.g., antisense oligonucleotides) and other non-peptide natural or synthetic compounds which specifically bind to the subject polynucleotide or polypeptide. Mutant forms of the subject polynucleotide which alter the enzymatic activity of the subject polypeptide or its localization in a cell are also contemplated. Crystallization of recombinant subject polypeptides alone and bound to a modulator, analysis of atomic structure by X-ray crystallography, and computer modeling of those structures are methods useful for designing and optimizing non-peptide selective modulators. See, for example, Erickson et al., 1992, *Ann. Rep. Med. Chem.*, 27: 271-289 for a general review of structure-based drug design.

Targets for the development of selective modulators include, for example: (1) the regions of the subject elongases which contact other proteins and/or localize the proteins within a cell, (2) the regions of the proteins which bind substrate, and (3) the phosphorylation site(s) of the subject polypeptides.

Thus, the present invention provides methods for screening and selecting compounds which promote disorders affected by lipids. As well, the present invention provides methods for screening and selecting compounds which treat or inhibit progression of diseases associated with lipid metabolism, such as eczema, cardiovascular disorders (including but not limited to hypertriglyceridemia, dyslipidemia, atherosclerosis, coronary artery disease, cerebrovascular disease and peripheral vascular disease), inflammation (including but not limited to sinusitis, asthma, pancreatitis, osteoarthritis, rheumatoid arthritis and acne), body weight disorders (including but not limited to obesity, cachexia and anorexia), psychiatric disorders, cancer, cystic fibrosis, pre-menstrual syndrome, diabetes and diabetic complications, and other diseases not necessary related to lipid metabolism.

*Protein Interaction Assays for DNA control regions, CDS and RNA of Elongase Genes.*

Protein interaction is implicated in virtually every biological process in the cell, for example, metabolism, transport, signaling and disease. Development of the yeast 2-hybrid and 1-hybrid systems have made it possible to study and identify protein-protein interaction, protein-DNA interaction or protein-RNA interaction *in vivo* (Fields S. and Song O., 1989, *Nature*, 340: 245-246; Ulmasov et al., 1997, *Science*, 276: 1865-1868; Furuyama K. and Sassa S., 2000, *J. Clin. Invest.*, 105: 757-764 and Gyuris et al., 1993, *Cell*, 75: 791-803). Because these interactions are key to cellular functions, identification of interacting partners is the first step towards elucidation of function and involvement in pathogenesis. New chemical entities that modulate (inhibit or activate) such interactions may have strong pharmaceutical and therapeutic benefit



in human, animal as well as plant diseases. It is now known that in sideroblastic anemic patients, the interaction between succinyl-CoA synthetase and the heme biosynthetic enzyme  $\delta$ -aminolevulinate synthase-E (ALAS-E) is disrupted (Furuyama K. and Sassa S., 2000, *J. Clin. Invest.*, 105: 757-764). Inhibition of gene expression in human cells through small molecule-RNA interaction have been recently described (Hwang et al., 1999, *Proc. Natl. Acad. Sci.*, 96: 12997-13002). The use of protein-RNA inhibition technology is a potential approach for development of anti-HIV therapeutics (Hamy et al., 1997, *Proc. Natl. Acad. Sci.*, 94: 3548-3553 and Mei et al., 1998, *Biochemistry*, 37: 14204-14212).

## 10 *Drug Design*

Antagonists and agonists and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds. The pharmaceutical compositions may be administered in any effective, convenient manner.

15 including, for instance, administration by direct microinjection into the affected area, or by intravenous or other routes. These compositions of the present invention may be employed in combination with a non-sterile or sterile carrier or carriers for use with cells, tissues or organisms, such as a pharmaceutical carrier suitable for administration to a subject. Such compositions comprise, for instance, a medium additive or a therapeutically effective amount  
20 of antagonists or agonists of the invention and a pharmaceutically acceptable carrier or excipient. Such carriers may include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol and combinations thereof. The formulation is prepared to suit the mode of administration.

25 Modulation of elongase gene function can be accomplished by the use of therapeutic agents or drugs which can be designed to interact with different aspects of elongase structure or function. For example, a drug or antibody can bind to a structural fold of the protein to correct a defective structure. Alternatively, a drug might bind to a specific functional residue and increase its affinity for a substrate or cofactor. Efficacy of a drug or agent can be identified by  
30 a screening program in which modulation is monitored *in vitro* in cell systems in which a defective elongase is expressed.

Alternatively, drugs can be designed to modulate the activity of proteins of elongase genes from knowledge of the structure and function correlations for such proteins and from  
35 knowledge of the specific defect in various mutant proteins (Copsey et al., 1988, *Genetically Engineered Human Therapeutic Drugs*, Stockton Press, NY).

*Gene Therapy*

5 A variety of gene therapy approaches may be used in accordance with the invention to modulate expression of the subject polynucleotides *in vivo*. For example, antisense DNA molecules may be engineered and used to block translation of mRNA of the subject polynucleotides *in vivo*. Alternatively, ribozyme molecules may be designed to cleave and destroy the mRNA of the subject polynucleotides *in vivo*. In another alternative, oligonucleotides designed to hybridize to the 5' region of the subject polynucleotide (including  
10 the region upstream of the coding sequence) and form triple helix structures may be used to block or reduce transcription of the subject polynucleotide. In yet another alternative, nucleic acid encoding the full length wild-type subject polynucleotide may be introduced *in vivo* into cells which otherwise would be unable to produce the wild-type subject polynucleotide product in sufficient quantities or at all.

15 For example, in conventional replacement therapy, gene product or its functional equivalent is provided to the patient in therapeutically effective amounts. Elongases can be purified using conventional techniques such as those described in Deutcher M. (ed.), 1990, *Guide to Protein Purification, Meth. Enzymol.*, Vol. 182. Sufficient amounts of gene product or protein for  
20 treatment can be obtained, for example, through cultured cell systems or synthetic manufacture. Drug therapies which stimulate or replace the gene product can also be employed. Delivery vehicles and schemes can be specifically tailored to the particular target gene.

25 Gene therapy using recombinant technology to deliver the gene into the patient's cells, or vectors which will supply the patient with gene product *in vivo*, is also within the scope of the invention. Retroviruses have been considered preferred vectors for experiments in somatic gene therapy, with a high efficiency of infection and stable integration and expression (Orkin et al., 1988, *Prog. Med. Genet.*, 7: 130-142). For example, elongase cDNAs can be cloned into  
30 a retroviral vector and driven from either its endogenous promoter or from the retroviral LTR (long terminal repeat). Other delivery systems which can be utilized include adeno-associated virus (McLaughlin et al., 1988, *J. Virol.*, 62: 1963-1973), vaccinia virus (Moss et al., 1987, *Annu. Rev. Immunol.*, 5: 305-324), bovine papilloma virus (Rasmussen et al., 1987, *Meth. Enzymol.*, 139: 642-654), or a member of the herpes virus group such as Epstein-Barr virus  
35 (Margolskee et al., 1988, *Mol. Cell. Biol.*, 8: 2837-2847).

Antisense, ribozyme and triple helix nucleotides are designed to inhibit the translation or transcription of the subject polynucleotides. To accomplish this, the oligonucleotides used should be designed on the basis of relevant sequences unique to the subject polynucleotides.

- 5 For example, and not by way of limitation, the oligonucleotides should not fall within those regions where the nucleotide sequence of a subject polynucleotide is most homologous to that of other polynucleotides, herein referred to as "unique regions".

- 10 In the case of antisense molecules, it is preferred that the sequence be chosen from the unique regions. It is also preferred that the sequence be at least 18 nucleotides in length in order to achieve sufficiently strong annealing to the target mRNA sequence to prevent translation of the sequence (Izant J.G. and Weintraub H., 1984, *Cell*, 36: 1007-1015 and Rosenberg et al., 1985, *Nature*, 313: 703-706).

- 15 In the case of the "hammerhead" type of ribozymes, it is also preferred that the target sequences of the ribozymes be chosen from the unique regions. Ribozymes are RNA molecules which possess highly specific endoribonuclease activity. Hammerhead ribozymes comprise a hybridizing region which is complementary in nucleotide sequence to at least part of the target RNA, and a catalytic region which is adapted to cleave the target RNA. The  
20 hybridizing region contains 9 or more nucleotides. Therefore, the hammerhead ribozymes of have a hybridizing region which is complementary to the sequences listed above and is at least nine nucleotides in length. The construction and production of such ribozymes are well known in the art and are described more fully in Haseloff J. and Gerlach W.L., 1988, *Nature*, 334: 585-591.

- 25 The ribozymes also include RNA endoribonucleases (hereinafter "Cech-type ribozymes") such as the one which occurs naturally in *Tetrahymena thermophila* (known as the IVS, or L-19 IVS RNA) and which has been extensively described by Thomas Cech and collaborators (Zaug et al., 1984, *Science*, 224: 574-578; Zaug A.J. and Cech T.R., 1986, *Science*, 231: 470-  
30 475; Zaug et al., 1986, *Nature*, 324: 429-433; Patent Publication Treaty International Patent Application No. WO 88/04300 and Been M.D. and Cech T.R., 1986, *Cell*, 47: 207-216). The Cech endoribonucleases have an eight base pair active site which hybridizes to a target RNA sequence whereafter cleavage of the target RNA takes place. Cech-type ribozymes target eight  
35 base-pair active site sequences are present in a subject polynucleotide but not other polynucleotides for elongases.

The compounds can be administered by a variety of methods which are known in the art, including, but not limited to the use of liposomes as a delivery vehicle. Naked DNA or RNA molecules may also be used where they are in a form which is resistant to degradation, such as by modification of the ends, by the formation of circular molecules, or by the use of alternate bonds including phosphothionate and thiophosphoryl modified bonds. In addition, the delivery of nucleic acid may be by facilitated transport where the nucleic acid molecules are conjugated to polylysine or transferrin. Nucleic acid may also be transported into cells by any of the various viral carriers, including but not limited to, retrovirus, vaccinia, adeno-associated virus, and adenovirus.

Alternatively, a recombinant nucleic acid molecule which encodes, or is, such antisense, ribozyme, triple helix, or subject polynucleotide molecule can be constructed. This nucleic acid molecule may be either RNA or DNA. If the nucleic acid encodes an RNA, it is preferred that the sequence be operatively attached to a regulatory element so that sufficient copies of the desired RNA product are produced. The regulatory element may permit either constitutive or regulated transcription of the sequence. A transfer vector such as a bacterial plasmid or viral RNA or DNA, encoding one or more of the RNAs, may be transfected into cells or cells of an organism (Llewellyn et al., 1987, *J. Mol. Biol.*, 195: 115-123 and Hanahan et al., 1983, *J. Mol. Biol.*, 166: 557-580). Once inside the cell, the transfer vector may replicate, and be transcribed by cellular polymerases to produce the RNA or it may be integrated into the genome of the host cell. Alternatively, a transfer vector containing sequences encoding one or more of the RNAs may be transfected into cells or introduced into cells by way of micromanipulation techniques such as microinjection, such that the transfer vector or a part thereof becomes integrated into the genome of the host cell.

#### *Composition, Formulation, and Administration of Pharmaceutical Compositions*

The pharmaceutical compositions of the present invention may be manufactured in a manner that is itself known, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. Pharmaceutical preparations for oral use can be obtained by solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol, or cellulose preparations such as, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone. If desired, disintegrating agents may be added, such as the cross-linked polyvinylpyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges (e.g. gelatin) for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multidose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for

example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

5 A pharmaceutical carrier for the hydrophobic compounds of the invention is a co-solvent system comprising benzyl alcohol, a nonpolar surfactant, a water-miscible organic polymer, and an aqueous phase. Naturally, the proportions of a co-solvent system may be varied considerably without destroying its solubility and toxicity characteristics. Furthermore, the identity of the co-solvent components may be varied.

10

Alternatively, other delivery systems for hydrophobic pharmaceutical compounds may be employed. Liposomes and emulsions are well known examples of delivery vehicles or carriers for hydrophobic drugs. Certain organic solvents such as dimethylsulfoxide also may be employed, although usually at the cost of greater toxicity. Additionally, the compounds may  
15 be delivered using a sustained-release system, such as semipermeable matrices of solid hydrophobic polymers containing therapeutic agent. Various sustained-release materials have been established and are well known by those skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the compounds for a few weeks up to over 100 days. Depending on the chemical nature and the biological stability of therapeutic reagent,  
20 additional strategies for protein stabilization may be employed.

The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include, but are not limited to, calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and  
25 polymers such as polyethylene glycols.

Many of the compounds of the invention may be provided as salts with pharmaceutically compatible counterions. Pharmaceutically compatible salts may be formed with many acids, including but, not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc.  
30 Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms.

Suitable routes of administration may, for example, include oral, rectal, transmucosal, transdermal, or intestinal administration; or parenteral delivery, including intramuscular,  
35 subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections.

Alternately, one may administer the compound in a local rather than systemic manner, for example, via injection of the compound directly into an affected area, often in a depot or sustained release formulation.

- 5 Furthermore, one may administer the drug in a targeted drug delivery system, for example, in a liposome coated with an antibody specific for affected cells. The liposomes will be targeted to and taken up selectively by the cells.

- 10 The pharmaceutical compositions generally are administered in an amount effective for treatment or prophylaxis of a specific indication or indications. It is appreciated that optimum dosage will be determined by standard methods for each treatment modality and indication, taking into account the indication, its severity, route of administration, complicating conditions and the like. In therapy or as a prophylactic, the active agent may be administered to an individual as an injectable composition, for example, as a sterile aqueous dispersion,
- 15 preferably isotonic. A therapeutically effective dose further refers to that amount of the compound sufficient to result in amelioration of symptoms associated with such disorders. Techniques for formulation and administration of the compounds of the instant application may be found in "*Remington's Pharmaceutical Sciences*," Mack Publishing Co., Easton, Pa., latest edition. For administration to mammals, and particularly humans, it is expected that the
- 20 daily dosage level of the active agent will be from 0.001 mg/kg to 10 mg/kg, typically around 0.01 mg/kg. The physician in any event will determine the actual dosage which will be most suitable for an individual and will vary with the age, weight and response of the particular individual. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the
- 25 scope of this invention.

The compounds of the invention may be particularly useful in animal disorders (veterinarian indications), and particularly mammals.

- 30 The invention further provides diagnostic and pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, reflecting approval by the agency of the manufacture,
- 35 use or sale of the product for human administration.



## DEFINITIONS

To facilitate a complete understanding of the invention, the terms defined below have the following meaning:

5

**Agonist** refers to any molecule or pharmaceutical agent, such as a drug or hormone, which enhances the activity of another molecule.

10

**Antagonist** refers to any molecule or pharmaceutical agent, such as a drug or hormone, which inhibits or extinguishes the activity of another molecule.

15

**Chemical Derivative.** As used herein, a molecule is said to be a "chemical derivative" of another molecule when it contains additional chemical moieties not normally a part of the molecule. Such moieties can improve the molecule's solubility, absorption, biological half life, and the like. The moieties can alternatively decrease the toxicity of the molecule, eliminate or attenuate any undesirable side effect of the molecule, and the like. Moieties capable of mediating such effects are disclosed in Mack E.W., 1990, *Remington's Pharmaceutical Sciences*, Mack Publishing Company, Easton, Pa., 13<sup>th</sup> edition. Procedures for coupling such moieties to a molecule are well known in the art.

20

**Compositions** include genes, proteins, polynucleotides, peptides, compounds, drugs, and pharmacological agents.

25

**Control region** refers to a nucleic acid sequence capable of, or required for, assisting or impeding initiation, termination, or otherwise regulating the transcription of a gene. The control region may include a promoter, enhancer, silencer and/or any other regulatory element. A control region also includes a nucleic acid sequence that may or may not be independently or exclusively sufficient to initiate, terminate, or otherwise regulate transcription, however, is capable of effecting such regulation in association with other nucleic acid sequences.

30

**Desaturase** refers to a fatty acid desaturase, which is an enzyme capable of generating a double bond in the hydrocarbon region of a fatty acid molecule.

35

**Disorder** as used herein refers to derangement or abnormality of structure or function. Disorder includes disease.

**Drug.** Drugs include, but are not limited to proteins, peptides, degenerate peptides, agents purified from conditioned cell medium, organic molecules, inorganic molecules, antibodies or oligonucleotides. The drug can be naturally occurring or synthetically or recombinantly produced.

**Enhancer** is a nucleic acid sequence comprising a DNA regulatory element that enhances or increases transcription when bound by a specific transcription factor or factors. Moreover, an enhancer may function in either orientation and in any location (upstream or downstream relative to the promoter) to effect and generate increased levels of gene expression when bound by specific factors. In addition, according to the present invention, an enhancer also refers to a compound (i.e. test compound) that increases or promotes the enzymatic activity of the elongase gene, and/or increases or promotes the transcription of the gene.

**Fatty Acids** are a class of compounds comprising a long saturated or mono or polyunsaturated hydrocarbon chain and a terminal carboxyl group.

**Fatty Acid Delta-5-Desaturase (D5D)** is an enzyme capable of generating a double bond between carbons 5 and 6 from the carboxyl group in a fatty acid molecule.

**Fatty Acid Delta-6-Desaturase** is an enzyme capable of generating a double bond between carbons 6 and 7 from the carboxyl group in a fatty acid molecule.

**Fatty Acid Elongase** is an enzyme required for the addition of an acetyl group or a 2-carbon chain to the carboxyl end of a fatty acid.

**Functional Enzyme**, as used herein, refers to a biologically active or non-active protein with a known enzymatic activity.

**Functional Derivative.** A "functional derivative" of a sequence, either protein or nucleic acid, is a molecule that possesses a biological activity (either functional or structural) that is substantially similar to a biological activity of the protein or nucleic acid sequence. A functional derivative of a protein can contain post-translational modifications such as covalently linked carbohydrate, depending on the necessity of such modifications for the performance of a specific function. The term "functional derivative" is intended to include the "fragments," "sequences," "variants," "analogs," or "chemical derivatives" of a molecule.

Gene refers to a nucleic acid molecule or a portion thereof, the sequence of which includes information required for the production of a particular protein or polypeptide chain. The polypeptide can be encoded by a full-length sequence or any portion of the coding sequence, so long as the functional activity of the protein is retained. A gene may comprise regions  
5 preceding and following the coding region as well as intervening sequences (introns) between individual coding sequences (exons). A "heterologous" region of a nucleic acid construct (i.e. a heterologous gene) is an identifiable segment of DNA within a larger nucleic acid construct that is not found in association with the other genetic components of the construct in nature. Thus, when the heterologous gene encodes a mammalian elongase gene, the gene will usually  
10 be flanked by a promoter that does not flank the structural genomic DNA in the genome of the source organism.

Host system may comprise a cell, tissue, organ, organism or any part thereof, which provides an environment or conditions that allow for, or enable, transcription and/or transcription.  
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Identity, similarity, homology or homologous, refer to relationships between two or more polynucleotide sequences, as determined by comparing the sequences. In the art, identity also means the degree of sequence relatedness between polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. Both identity and similarity  
20 can be readily calculated (Lesk A.M., ed., 1988, *Computational Molecular Biology*, Oxford University Press, NY; Smith D.W., ed., 1993, *Biocomputing: Informatics and Genome Project*, Academic Press, NY; Griffin A.M. and Griffin H.G., eds., 1994, *Computer Analysis of Sequence Data, Part 1*, Humana Press, NJ; von Heijne G., 1987, *Sequence Analysis in Molecular Biology*, Academic Press, NY and Gribskov M. and Devereux J., eds., 1991, *Sequence Analysis Primer*, M Stockton Press, NY). While there exist a number of methods to measure identity and similarity between two polynucleotide sequences, both terms are well known to skilled artisans (von Heijne G., 1987, *Sequence Analysis in Molecular Biology*, Academic Press, NY; Gribskov M. and Devereux J., eds., 1991, *Sequence Analysis Primer*, M Stockton Press, NY and Carillo H. and Lipman D., 1988, *SIAM J. Applied Math.*, 48: 1073).  
30 Methods commonly employed to determine identity or similarity between sequences include, but are not limited to those disclosed in Carillo H. and Lipman D., 1988, *SIAM J. Applied Math.*, 48: 1073. Methods to determine identity and similarity are codified in computer programs. Computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCG program package (Devereux et al., 1984, *Nucl. Acid Res.*, 12: 387-395), BLASTP, BLASTN and FASTA (Altschul et al., 1990, *J. Molec. Biol.*, 215: 403-410).  
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**Isolated** means altered "by the hand of man" from its natural state; i.e., that, if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a naturally occurring polynucleotide naturally present in a living organism in its natural state is not "isolated," but the same polynucleotide separated from coexisting materials of its natural state is "isolated", as the term is employed herein. As part of or following isolation, such polynucleotides can be joined to other polynucleotides, such as DNA, for mutagenesis, to form fusion proteins, and for propagation or expression in a host, for instance. The isolated polynucleotides, alone or joined to other polynucleotides such as vectors, can be introduced into host cells, in culture or in whole organisms. Introduced into host cells in culture or in whole organisms, such DNA still would be isolated, as the term is used herein, because they would not be in their naturally occurring form or environment. Similarly, the polynucleotides may occur in a composition, such as a media formulations, solutions for introduction of polynucleotides, for example, into cells, compositions or solutions for chemical or enzymatic reactions, for instance, which are not naturally occurring compositions, and, therein remain isolated polynucleotides within the meaning of that term as it is employed herein.

**Mutation.** A "mutation" is any detectable change in the genetic material. A mutation can be any (or a combination of) detectable, unnatural change affecting the chemical or physical constitution, mutability, replication, phenotypic function, or recombination of one or more deoxyribonucleotides; nucleotides can be added, deleted, substituted for, inverted, or transposed to new positions with and without inversion. Mutations can occur spontaneously and can be induced experimentally by application of mutagens or by site-directed mutagenesis. A mutant polypeptide can result from a mutant nucleic acid molecule.

**Nucleic acid construct** refers to any genetic element, including, but not limited to, plasmids and vectors, that incorporate polynucleotide sequences. For example, a nucleic acid construct may be a vector comprising a promoter or control region that is operably linked to a heterologous gene.

**Operably linked** as used herein indicates the association of a promoter or control region of a nucleic acid construct with a heterologous gene such that the presence or modulation of the promoter or control region influences the transcription of the heterologous gene, including genes for reporter sequences. Operably linked sequences may also include two segments that are transcribed onto the same RNA transcript. Thus, two sequences, such as a promoter and a "reporter sequence" are operably linked if transcription commencing in the promoter produces an RNA transcript of the reporter sequence.

**Plasmids.** Starting plasmids disclosed herein are either commercially available, publicly available, or can be constructed from available plasmids by routine application of well known, published procedures. Many plasmids and other cloning and expression vectors that can be  
5 used in accordance with the present invention are well known and readily available to those of skill in the art. Moreover, those of skill readily may construct any number of other plasmids suitable for use in the invention.

**Polynucleotides(s)** of the present invention may be in the form of RNA, such as mRNA, or in  
10 the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced by chemical synthetic techniques or by a combination thereof. The DNA may be double-stranded or single-stranded. Single-stranded polynucleotides may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand. Polynucleotides generally refers to any polyribonucleotide or  
15 polydeoxiribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. Thus, for instance, polynucleotides as used herein refers to, among others, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions or single-, double- and triple-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA  
20 that may be single-stranded or, more typically, double-stranded, or triple-stranded, or a mixture of single- and double-stranded regions. In addition, polynucleotide as used herein refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The strands in such regions may be from the same molecule or from different molecules. The regions may include all of one or more of the molecules, but more typically involve only a region of some  
25 of the molecules. One of the molecules of a triple-helical region often is an oligonucleotide. As used herein, the term polynucleotide also includes DNA or RNA that contain one or more modified bases. Thus, DNA or RNA with backbones modified for stability or for other reasons are "polynucleotides" as that term is intended herein. Moreover, DNA or RNA comprising unusual bases, such as inosine, or modified bases, such as tritylated bases, to name just two  
30 examples, are polynucleotides as the term is used herein. It will be appreciated that a great variety of modifications have been made to DNA and RNA that serve many useful purposes known to those of skill in the art. The term polynucleotide as it is employed herein embraces such chemically, enzymatically or metabolically modified forms of polynucleotides, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including simple and  
35 complex cells, *inter alia*. Polynucleotides embraces short polynucleotides often referred to as oligonucleotide(s). It will also be appreciated that RNA made by transcription of this doubled stranded nucleotide sequence, and an antisense strand of a nucleic acid molecule of the

invention or an oligonucleotide fragment of the nucleic acid molecule, are contemplated within the scope of the invention. An antisense sequence is constructed by inverting the sequence of a nucleic acid molecule of the invention, relative to its normal presentation for transcription. Preferably, an antisense sequence is constructed by inverting a region preceding the initiation codon or an unconserved region. The antisense sequences may be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art.

**Promoter** refers to a nucleic acid sequence comprising a DNA regulatory element capable of binding RNA polymerase directly or indirectly to initiate transcription of a downstream (3' direction) gene. In accordance with the present invention, a promoter of a nucleic acid construct that includes a nucleotide sequence, wherein the nucleotide sequence may be linked to a heterologous gene such that the induction of the promoter influences the transcription of the heterologous gene.

**Purified:** A "purified" protein or nucleic acid is a protein or nucleic acid preparation that is generally free of contaminants, whether produced recombinantly, chemically synthesized or purified from a natural source.

**Recombinant** refers to recombined or new combinations of nucleic acid sequences, genes, or fragments thereof which are produced by recombinant DNA techniques and are distinct from a naturally occurring nucleic acid sequence

**Regulatory element** refers to a deoxyribonucleotide sequence comprising the whole, or a portion of, a nucleic acid sequence to which an activated transcriptional regulatory protein, or a complex comprising one or more activated transcriptional regulatory proteins, binds so as to transcriptionally modulate the expression of an associated gene or genes, including heterologous genes.

**Reporter gene** is a nucleic acid coding sequence whose product is a polypeptide or protein that, is not otherwise produced by the host cell or host system, or which is produced in minimal or negligible amounts in the host cell or host system, and which is detectable by various known methods such that the reporter gene product may be quantitatively assayed to analyse the level of transcriptional activity in a host cell or host system. Examples include genes for luciferase, chloramphenicol acetyl transferase (CAT), beta-galactosidase, secreted placental alkaline phosphatase and other secreted enzymes.

**Silencer** refers to a nucleic acid sequence or segment of a DNA control region such that the presence of the silencer sequence in the region of a target gene suppresses the transcription of the target gene at the promoter through its actions as a discrete DNA segment or through the actions of trans-acting factors that bind to these genetic elements and consequently effect a negative control on the expression of a target gene.

**Stringent hybridization conditions** are those which are stringent enough to provide specificity, reduce the number of mismatches and yet are sufficiently flexible to allow formation of stable hybrids at an acceptable rate. Such conditions are known to those skilled in the art and are described, for example, in Sambrook et al., 1989, *Molecular Cloning, 2nd Edition*, Cold Spring Harbor Laboratory Press, Cold Spring Harbour, NY or Ausubel et al., 1994-, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY. By way of example only, stringent hybridization with short nucleotides may be carried out at 5-10°C below the  $T_M$  using high concentrations of probe such as 0.01-1.0 pmole/ml. Preferably, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences.

**Tag** refers to a specific short amino acid sequence, or the oligonucleotide sequence that encodes it, wherein said amino acid or nucleic acid sequence may comprise or encode, for example, a c-myc epitope and/or a string of six histidine residues recognizable by commercially available antibodies. In practice, a tag facilitates the subsequent identification and purification of a tagged protein.

**Tagged protein** as used herein refers to a protein comprising a linked tag sequence. For example, a tagged protein includes a mammalian elongase polypeptide linked to a c-myc epitope and six histidine residues at the carboxyl terminus of the amino acid sequence.

**Test compounds** as used herein encompass small molecules (e.g. small organic molecules), pharmacological compounds or agents, peptides, proteins, antibodies or antibody fragments, and nucleic acid sequences, including DNA and RNA sequences.

**Transfection** refers to a process whereby exogenous or heterologous DNA (i.e. a nucleic acid construct) is introduced into a recipient eukaryotic host cell. Therefore, in eukaryotic cells, the acquisition of exogenous DNA into a host cell is referred to as transfection. In prokaryotes and eukaryotes (for example, yeast and mammalian cells) introduced DNA may be maintained on an episomal element such as a plasmid or integrated into the host genome. With respect to eukaryotic cells, a stably transfected cell is one in which the introduced DNA has become

integrated into a chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones comprised of a population of daughter cells containing the introduced DNA.

5     **Transformation** refers to a process whereby exogenous or heterologous DNA (i.e. a nucleic acid construct) is introduced into a recipient prokaryotic host cell. Therefore, in prokaryotic cells, the acquisition of exogenous DNA into a host cell is referred to as transformation.

Transformation in eukaryotes refers to the conversion or transformation of eukaryotic cells to a state of unrestrained growth in culture, resembling a tumorigenic condition. In prokaryotes and eukaryotes (for example, yeast and mammalian cells) introduced DNA may be maintained  
10     on an episomal element such as a plasmid or integrated into the host genome. With prokaryotic cells, a stably transformed bacterial cell is one in which the introduced DNA has become integrated into a chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the prokaryotic cell to establish cell  
15     lines or clones comprised of a population of daughter cells containing the introduced DNA.

**Transfection/transformation** as used herein refers to a process whereby exogenous or heterologous DNA (e.g. a nucleic acid construct) has been introduced into a eukaryotic or prokaryotic host cell or into a host system.

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**Variant(s)** of polynucleotides are polynucleotides that differ in nucleotide sequence from another, reference polynucleotide. A "variant" of a protein or nucleic acid is meant to refer to a molecule substantially similar in structure and biological activity to either the protein or nucleic acid. Thus, provided that two molecules possess a common activity and can substitute  
25     for each other, they are considered variants as that term is used herein even if the composition or secondary, tertiary, or quaternary structure of one of the molecules is not identical to that found in the other, or if the amino acid or nucleotide sequence is not identical. Generally, differences are limited so that the nucleotide sequences of the reference and the variant are closely similar overall and, in many regions, identical. Changes in the nucleotide sequence of  
30     the variant may be silent. That is, they may not alter the amino acids encoded by the polynucleotide. Where alterations are limited to silent changes of this type a variant will encode a polypeptide or polynucleotide with the same amino acid sequence as the reference. Changes in the nucleotide sequence of the variant may alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Such nucleotide changes may result in  
35     amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide or polynucleotide encoded by the reference sequence.



**Vector.** A plasmid or phage DNA or other DNA sequence into which DNA can be inserted to be cloned. The vector can replicate autonomously in a host cell, and can be further characterized by one or a small number of endonuclease recognition sites at which such DNA sequences can be cut in a determinable fashion and into which DNA can be inserted. The vector can further contain a marker suitable for use in the identification of cells transformed with the vector. Markers, for example, are tetracycline resistance or ampicillin resistance. The words "cloning vehicle" are sometimes used for "vector."

The terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs.

The present invention is further described and will be better understood by referring to the working examples set forth below. These non-limiting examples are to be considered illustrative only of the principles of the invention. Since numerous modifications and changes will readily occur to those skilled in the art, it is not desired to limit the invention to the exact construction and operation shown and described. Accordingly, all suitable modifications and equivalents may be used and will fall within the scope of the invention and the appended claims.

## EXAMPLES

The present invention is further described by the following examples. These examples, while illustrating certain specific aspects of the invention, do not portray the limitations or circumscribe the scope of the disclosed invention.

### Example 1- Cloning ELG1

ELG1 was cloned into the pYES2/CT yeast expression vector (Invitrogen) using PCR. Two plasmid constructions were made for the production of the ELG1 protein with either a C-terminal tag containing the V-5 epitope and polyhistidine peptide (ELG1/V5-His), or the ELG1 protein without the tag (ELG1). The forward primer (5'-CACGCGGGTACCAGGATGGAGGCTGTTGTGAAC-3') contains the translation start codon and a *KpnI* site (underlined). The reverse primers for cloning ELG1 and ELG1/V5-His,

5'-ATATCACGATGCGGCCGCTCAGTTGGCCTTGACCTTGGC-3' and 5'-  
ATATCACGATGCGGCCGCCAGTTGGCCTTGACCTTGGC-3', respectively, contain a  
*NotI* site (underlined). The reverse primer for cloning ELG1 provides the translation stop  
codon. The reverse primer for cloning ELG1/V5-His only contains 2 of the 3 bases of the stop  
5 codon, therefore, placing the gene in frame with the tag provided by the vector.

PCR was carried out using Advantage-HF polymerase (Clontech) as per the manufacturer's  
instructions. The SuperScript human leukocyte cDNA library (Gibco BRL) was used as the  
DNA template for cloning ELG1. pTh1009.1 (defined below) was used as the template for  
10 cloning ELG1/V5-His.

The PCR products were gel purified, digested with *KpnI* and *NotI*, and ligated into pYES2/CT  
cut with the same enzymes. The ligation products were used to transform *E. coli* strain INV $\alpha$ F'  
(Invitrogen). Plasmids were isolated and their inserts were sequenced. Plasmids coding for  
15 ELG1 and ELG1/V5-His were designated pTh1009.1 (Figure 7) and pTh1009.2 (Figure 18),  
respectively.

### Example 2 - Cloning ELG2

#### Obtaining Complete Coding Sequence for ELG2

20 Clones containing the complete coding sequence for ELG2 were obtained from the  
SuperScript human leukocyte cDNA library (Gibco BRL) using the GeneTrapper cDNA  
Positive Selection System (Gibco BRL) as per the manufacturer's instructions. The sequence  
of the oligonucleotide used to probe the library and repair the captured cDNA target was 5'-  
GTAACAGGAGTATGGGAAGGCA-3'. The repaired DNA was used to transform UltraMax  
25 DH5 $\alpha$ -FT cells (Gibco BRL). Clones containing ELG2 were identified by colony PCR using  
5'-TTGGACTCACACTGCTGTCTCT-3' and 5'-GTGTGGCACCAAAATAAGAGTG-3' as  
gene specific primers and Platinum Taq DNA polymerase (Gibco BRL). Plasmid DNA was  
isolated from selected colonies and their inserts were sequenced. The nucleotide sequence  
obtained was used to identify the open reading frame for ELG2 and to design primers for  
30 cloning ELG2 into a yeast expression vector. A plasmid containing the complete ELG2 coding  
sequence was designated pSh1010.1.

#### Cloning ELG2 into Expression Vector

ELG2 was cloned into the pYES2/CT yeast expression vector (Invitrogen) using PCR. Two  
35 plasmid constructions were made for the production of the ELG2 protein with either a C-

terminal tag containing the V-5 epitope and polyhistidine peptide (ELG2/V5-His), or the ELG2 protein without the tag (ELG2). The forward primer (5'-CACGCGGGATCCCAAATGGAACATTTTGATGCATCAC-3') contains the translation start codon and a *Bam*HI site (underlined). The reverse primers for cloning ELG2 and ELG2/V5-His, 5'-ATATCACGATGCGGCCGCTCAATCCTTCCGCAGCTTCC-3' and 5'-ATATCACGATGCGGCCGCCAATCCTTCCGCAGCTTCC-3', respectively, contain a *Not*I site (underlined). The reverse primer for cloning ELG2 provides the translation stop codon. The reverse primer for cloning ELG2/V5-His only contains 2 of the 3 bases of the stop codon, therefore, placing the gene in frame with the tag provided by the vector.

PCR was carried out using Advantage-HF polymerase (Clontech) as per the manufacturer's instructions. pSh1010.1 was used as the DNA template for cloning ELG2. pMr1014.1 (described below) was used as the DNA template for ELG2/V5-His.

The PCR products were gel purified, digested with *Bam*HI and *Not*I, and ligated into pYES2/CT cut with the same enzymes. The ligation products were used to transform *E. coli* strain TOP10F' (Invitrogen). Plasmids were isolated and their inserts were sequenced. Plasmids coding for ELG2 and ELG2/V5-His were designated pTh1014.1 and pTh1014.2, respectively.

### Example 3- Cloning ELG3

ELG3 was cloned into the pYES2/CT yeast expression vector (Invitrogen) using PCR. Two plasmid constructions were made for the production of the ELG3 protein with either a C-terminal tag containing the V-5 epitope and polyhistidine peptide (ELG3/V5-His), or the ELG3 protein without the tag (ELG3). The forward primer (5'-CACGCGGGATCCATCATGGAACATCTAAAGGCC-3') contains the translation start codon and a *Bam*HI site (underlined). The reverse primers for cloning ELG3 and ELG3/V5-His, 5'-ATATCACGATGCGGCCGCTTATTGTGCTTTCTTGTTTCATCACTCC-3' and 5'-ATATCACGATGCGGCCGCTTTTGTGCTTTCTTGTTTCATCACTCC-3', respectively, contain a *Not*I site (underlined). The reverse primer for cloning ELG3 provides the translation stop codon. The reverse primer for cloning ELG3/V5-His only contains 2 of the 3 bases of the stop codon, therefore, placing the gene in frame with the tag provided by the vector.

PCR was carried out using Advantage-HF polymerase (Clontech) as per the manufacturer's instructions. cDNA prepared from ZR-75-1 cells (ATCC No. CRL-1500) was used as the

DNA template. This cDNA was prepared by isolating RNA from the ZR-75-1 cells using Trizol reagent (Gibco BRL) as per the manufacturer's instructions and then reverse transcribing the RNA using MuLV reverse transcriptase and random hexamers as described for the GeneAmp RNA PCR kit (PE Applied Biosystems).

5 PCR products were gel purified, digested with *Bam*HI and *Not*I, and ligated into pYES2/CT cut with the same enzymes. The ligation products were used to transform *E. coli* strain TOP10F' (Invitrogen). Plasmids were isolated and their inserts were sequenced. Plasmids coding for ELG3 and ELG3/V5-His were designated pTh1015.1 and pTh1017.1, respectively.

10 ELG3 was also cloned into the pBEVY-L yeast expression vector (Miller et al., 1998, *Nucl. Acids Res.*, 26: 3577-3583) under the control of the constitutive glyceraldehyde 3-phosphate dehydrogenase promoter. The ELG3 coding sequence was obtained by restricting pTh1015.1 with *Bam*HI and *Xba*I, and gel purifying the ~0.9 kb fragment. The pBEVY vector was  
15 restricted with *Bam*HI and *Eco*RI, or *Xba*I and *Eco*RI, and the ~1 kb and ~6 kb fragments, respectively, were gel purified. The three fragments were ligated and the ligation products were used to transform *E. coli* strain INV $\alpha$ F' (Invitrogen). A plasmid containing the ELG3 gene was isolated and identified by restriction analysis. The insert DNA was confirmed by DNA sequencing and the plasmid designated pLh5015.1 (Figure 19).

#### 20 Example 4 - Cloning ELG4

##### Obtaining Complete Coding Sequence for ELG4

A cDNA clone with an incomplete coding sequence for ELG4 was obtained from the  
25 SuperScript human leukocyte cDNA library (Gibco BRL) using the GeneTrapper cDNA Positive Selection System (Gibco BRL) as per the manufacturer's instructions. The sequence of the oligonucleotide used to probe the library and repair the captured cDNA target was 5'-GCCAGCCTACCAGAAGTATTTG-3'. The repaired DNA was used to transform UltraMax DH5 $\alpha$ -FT cells (Gibco BRL). A clone containing ELG4 was identified by colony PCR using  
30 5'-GCGCAAGAAAAATAGCCAAG-3' and 5'-AATGATGCACGCAAAGACTG-3' as gene specific primers and Platinum Taq DNA polymerase (Gibco BRL). Plasmid DNA was isolated and the insert was sequenced. The plasmid was designated pSh1026.1. The complete coding sequence for ELG4 could not be determined, however, an open reading frame containing the C-terminus of the ELG4 protein was identified. Subsequent cloning (described below)  
35 revealed that pSh1026.1 contains an ELG4 variant with an internal deletion of nucleotides 210-255 of the coding sequence.

The nucleotide sequence obtained from pSh1026.1 was used to design a forward (5'-CACGCGGGATCCCTGATGAATACAGAGCCGTGG-3') and reverse (5'-ATATCACGATGCGGCCGCTCAATTATCTTTGTTTTGCAAGTTCC-3') primer for cloning ELG4 by PCR. These primers contain a *Bam*HI and *Not*I site, respectively (underlined). The forward primer includes the first possible translation start codon identified in pSh1026.1. The reverse primer provides the translation stop codon.

PCR was carried out using Advantage HF polymerase (Clontech) as per the manufacturer's instructions. The SuperScript human leukocyte cDNA library (Gibco BRL) was used as the DNA template.

The PCR products were gel purified, digested with *Bam*HI and *Not*I, and ligated into pYES2/CT (Invitrogen) cut with the same enzymes. The ligation products were used to transform *E. coli* strain TOP10 (Invitrogen). Plasmids were isolated and their inserts were sequenced. A plasmid containing the complete coding sequence for ELG4 as well as 108 nucleotides of 5'-UTR was designated pTh1030.1.

#### Cloning ELG4 into Expression Vector

ELG4 was cloned into the pYES2/CT yeast expression vector using PCR. Two plasmid constructions were made for the production of the ELG4 protein with either a C-terminal tag containing the V-5 epitope and polyhistidine peptide (ELG4/V5-His), or the ELG4 protein without the tag (ELG4). The forward primer (5'-CACGCGGGATCCCTGATGGAAAAGCCCATTAATATTC-3') contains the translation start codon and a *Bam*HI site (underlined). The reverse primers for cloning ELG4 and ELG4/V5-His, 5'-ATATCACGATGCGGCCGCTCAATTATCTTTGTTTTGCAAGTTCC-3' and 5'-ATATCACGATGCGGCCGCCAATTATCTTTGTTTTGCAAGTTCC-3', respectively, contain a *Not*I site (underlined). The reverse primer for cloning ELG4 provides the translation stop codon. The reverse primer for cloning ELG4/V5-His only contains 2 of the 3 bases of the stop codon, therefore, placing the gene in frame with the tag provided by the vector.

PCR was carried out using Advantage-HF polymerase (Clontech) as per the manufacturer's instructions. pTh1030.1 was used as the DNA template for ELG4 and pTh1021.1 (described below) was used as the template for ELG4/V5-His.

The PCR products were gel purified, digested with *Bam*HI and *Not*I, and ligated into pYES2/CT cut with the same enzymes. The ligation products were used to transform *E. coli* strain TOP10 (Invitrogen). Plasmids were isolated and their inserts were sequenced. Plasmids coding for ELG4 and ELG4/V5-His were designated pTh1021.1 and pTh1021.2, respectively.

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#### **Example 5 - Cloning ELG5**

ELG5 was cloned into the pYES2/CT yeast expression vector (Invitrogen) using PCR. Two plasmid constructions were made for the production of the ELG5 protein with either a C-terminal tag containing the V-5 epitope and polyhistidine peptide (ELG5/V5-His), or the ELG5 protein without the tag (ELG5). The forward primer (5'-CACGCGGGATCCAAAAATGAACATGTCAGTGTGACTTTACAAG-3') contains the translation start codon and a *Bam*HI site (underlined). The reverse primers for cloning ELG5 and ELG5/V5-His, 5'-ATATCACGATGCGGCCGCTATTCAGCTTTCGTTGTTTCCTC-3' and 5'-ATATCACGATGCGGCCGCCATTCAGCTTTCGTTGTTTCCTC-3', respectively, contain a *Not*I site (underlined). The reverse primer for cloning ELG5 provides the translation stop codon. The reverse primer for cloning ELG5/V5-His only contains 2 of the 3 bases of the stop codon, therefore, placing the gene in frame with the tag provided by the vector.

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PCR was carried out using Advantage-HF polymerase (Clontech) as per the manufacturer's instructions. The ProQuest human liver cDNA library (Gibco BRL) was used as the DNA template.

25 The PCR products were gel purified, digested with *Bam*HI and *Not*I, and ligated into pYES2/CT cut with the same enzymes. The ligation products were used to transform *E. coli* strain TOP10 (Invitrogen). Plasmids were isolated and their inserts were sequenced. Plasmids coding for ELG5 and ELG5/V5-His were designated pTh1018.1 and pTh1019.1, respectively.

#### **Example 6 - Cloning ELG6**

ELG6 was cloned into the pYES2/CT yeast expression vector (Invitrogen) using PCR. Two plasmid constructions were made for the production of the ELG6 protein with either a C-terminal tag containing the V-5 epitope and polyhistidine peptide (ELG6/V5-His), or the ELG6 protein without the tag (ELG6). The forward primer (5'-CACGCGGGATCCAAAAATGGTCACAGCCATGAATGTCTC-3') contains the translation start codon and a *Bam*HI site (underlined). The reverse primers for cloning ELG6 and

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ELG6/V5-His, 5'-ATATCACGATGCGGCCGCTCACTGGCTCTTGGTCTTGGC-3' and 5'-ATATCACGATGCGGCCGCTCACTGGCTCTTGGTCTTGGC-3', respectively, contain a *NotI* site (underlined). The reverse primer for cloning ELG6 provides the translation stop codon. The reverse primer for cloning ELG6/V5-His only contains 2 of the 3 bases of the stop codon, therefore, placing the gene in frame with the tag provided by the vector.

PCR was carried out using Advantage-HF polymerase (Clontech) as per the manufacturer's instructions. The SuperScript human leukocyte cDNA library (Gibco BRL) was used as the DNA template.

The PCR products were gel purified, digested with *Bam*HI and *Not*I, and ligated into pYES2/CT cut with the same enzymes. The ligation products were used to transform *E. coli* strain TOP10 (Invitrogen). Plasmids were isolated and their inserts were sequenced. Plasmids coding for ELG6 and ELG6/V5-His were designated pTh1041.1 and pTh1042.1, respectively.

#### Example 7 - Cloning ELG7

ELG7 was cloned into the pYES2/CT yeast expression vector (Invitrogen) using PCR. Two plasmid constructions were made for the production of the ELG7 protein with either a C-terminal tag containing the V-5 epitope and polyhistidine peptide (ELG7/V5-His), or the ELG7 protein without the tag (ELG7). The forward primer (5'-CACGCGGGATCCAAAAATGGGGCTCCTGGACTCGGAGC-3') contains the translation start codon and a *Bam*HI site (underlined). The reverse primers for cloning ELG7 and ELG7/V5-His, 5'-ATATCACGATGCGGCCGCTTAATCTCCTTTTGCTTTTCCATTTTCTGC-3' and 5'-ATATCACGATGCGGCCGCTTATCTCCTTTTGCTTTTCCATTTTCTGC-3', respectively, contain a *Not*I site (underlined). The reverse primer for cloning ELG7 provides the translation stop codon. The reverse primer for cloning ELG7/V5-His only contains 2 of the 3 bases of the stop codon, therefore, placing the gene in frame with the tag provided by the vector.

PCR was carried out using Platinum Taq DNA polymerase (Gibco BRL) as per the manufacturer's instructions. The SuperScript human leukocyte cDNA library (Gibco BRL) was used as the DNA template.

The PCR products were gel purified, digested with *Bam*HI and *Not*I, and ligated into pYES2/CT cut with the same enzymes. The ligation products were used to transform *E. coli*

strain TOP10 (Invitrogen). Plasmids were isolated and their inserts were sequenced. Plasmids coding for ELG7 and ELG7/V5-His were designated pTh1044.1 and pTh1045.1, respectively.

#### **Example 8 - Determination of Tissue Distribution by Northern Blot Analysis**

5

A membrane containing poly(A)<sup>+</sup> RNA from 12 different human tissues (brain, heart, skeletal muscle, colon, thymus, spleen, kidney, liver, small intestine, placenta, lung and peripheral blood leukocytes) was purchased from Clontech (Human 12-lane MTN blot). Northern blot analysis was carried out using standard procedures (Ausubel et al., 1994-, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY). The hybridization solution contained 10% dextran sulphate. Probes were prepared by labelling cDNA using [alpha-<sup>32</sup>P]dCTP and Rediprime II Random Prime Labelling System (Amersham Pharmacia Biotech). The cDNA probes for ELG1, ELG3, ELG5, and ELG6 corresponded to the complete CDS for the genes. The cDNA probes for ELG2, ELG4 and ELG7 corresponded to bases 209-514, 408-726 and 113-566 of the CDS, respectively. The membrane was washed at high stringency using 0.25X SSC, 0.1% SDS at 55°C. The Northern blots are shown in Figure 27.

10  
15

#### **Example 9 - Cloning Human ELG1 Control Region**

The ELG1 control region (989 bp) is cloned from human leukocyte genomic DNA by PCR. The control region is amplified by PCR using synthetic forward and reverse primers starting at positions -2865 bp and -1877 bp upstream from the translation initiation codon, ATG. The forward and reverse primers used for cloning human ELG1 control region by PCR amplification are 5'-GGAAGATCTTACAGGCTCGTGAGGCTTCCCTCCCG-3' and 5'-GGAAGATCTCCGGCAGGAGGGACCAAGGCT-3', respectively. The *Bgl*III recognition sequence (underlined) is included to facilitate cloning.

20  
25

The PCR amplification is performed in a Perkin-Elmer GeneAMP PCR system 9700 instrument. For example, the PCR is performed in a 50 µl reaction volume containing 0.5 µg of genomic DNA, 0.4 µM of each primer, 1X dNTP mix (Clontech, CA), 1X cDNA PCR reaction buffer (Clontech) and 1X Advantage cDNA polymerase mix (Clontech).

30

The conditions for the PCR reaction are:

35

7 cycles at 94°C for 2 seconds, 72°C for 3 minutes  
32 cycles at 94°C for 2 seconds, 67°C for 3 minutes  
67°C for 4 minutes



The PCR product is gel-purified using QIAquick gel extraction kit (Qiagen, Germany) and ligated into the TA cloning vector pCRII (Invitrogen) according to manufacturers instruction. The ligation product is used to transform *E. coli* TOP10 strain (Invitrogen). The resulting plasmids are screened by restriction analysis and confirmed by DNA sequencing. The human ELG1 control region is then recloned from the pCRII/ELG1 control region construct into the luciferase reporter vector pGL3-Basic (Promega). The resulting human ELG1 control region/reporter construct is used to transfect different mammalian cell lines, and reporter activity measured.

#### 10 **Example 10 - Cloning Human ELG2 Control Region**

The ELG2 control region (509 bp) is cloned from human leukocyte genomic DNA by PCR. The control region is amplified by PCR using synthetic forward and reverse primers starting at positions -53626 bp and -53118 bp upstream from the translation initiation site, ATG. The forward and reverse primers used for cloning human ELG2 control region by PCR amplification are 5'-GGAAGATCTCGAGGGTGGGCTTCTGCCACCC-3' and 5'-GGAAGATCTCTTTTAGCCCAAGGGGCGGCAGC-3', respectively. The *Bgl*II recognition sequence (underlined) is included to facilitate cloning.

20 The PCR amplification and cloning are performed as described in Example 9.

The resulting human ELG2 control region/reporter construct is used to transfect different mammalian cell lines, and reporter activity measured.

#### 25 **Example 11 - Cloning of the Human ELG3 Control Region**

The human ELG3 control region was cloned from human leukocyte genomic DNA by nested PCR. Blood was obtained from volunteers in the present inventors' laboratory and used to prepare genomic DNA that served as template. In the first PCR reaction, synthetic forward and reverse primers starting at position -2025 bp and -1 bp, respectively, upstream from the translation initiation codon, ATG of the ELG3 gene were used. The forward and reverse primers were 5'-GGAAGATCTTTCGTGTGAATTCCTTCAAGTCTC-3' and 5'-GGAAGATCTTGATCCGCAGCGGCTGTG-3', respectively. The *Bgl*II recognition sequence (underlined) was included to facilitate cloning.

35

The PCR amplification was conducted in a Perkin-Elmer GeneAMP PCR system 9700 instrument, in a 50 µl reaction volume containing 0.5 µg of genomic DNA, 0.4 µM of each primer, 1X dNTP mix (Clontech, CA), 1X cDNA PCR reaction buffer (Clontech) and 1X Advantage cDNA polymerase mix (Clontech).

5

The conditions for the PCR reaction were:

7 cycles at 94°C for 2 seconds, 72°C for 3 minutes

32 cycles at 94°C for 2 seconds, 67°C for 3 minutes

10

67°C for 4 minutes

Analysis of the PCR product by agarose gel electrophoresis revealed that at least two primer specific bands of about 2 kb were amplified. This result necessitated the use of the PCR products as a template and a new set of internal primers in a second PCR reaction to generate a unique primer specific band corresponding to the ELG3 control region. The internal forward and reverse primers start at positions -1381 and -37 respectively, upstream from the translation initiation codon, ATG. The internal forward and reverse primers used were 5'-

15

GGAAGATCTCCGGTACCTACAGTTACTCACTCTGC-3' and 5'-

GGAAGATCTGGCGATGCGCTGTCCAGGGTA-3'.

20

The conditions for PCR reaction described herein were used for the second PCR reaction except for the following modifications: the second temperature cycle was lowered from 32 to 22 cycles, Taq DNA polymerase was substituted for cDNA polymerase and Q solution (Qiagen) was used according to manufacturer's instruction.

25

The PCR product was gel-purified using QIAquick gel extraction kit (Qiagen). The purified PCR product and the reporter vector pGL3-Basic were separately digested with *Bgl*II restriction enzyme to generate compatible ends suitable for in-frame ligation of the PCR product to the luciferase gene of pGL3-basic. The ligation product was used to transform *E. coli* TOP10 strain (Invitrogen). The resulting plasmid, pGh3020.1 (Figure 20), was screened by restriction analysis and confirmed by DNA sequencing. The resulting human ELG3 control region/reporter construct is used to transfect different mammalian cell lines, and reporter activity measured.

30

**Example 12 - Cloning Human ELG4 Control Region**

The ELG4 control region (2456 bp) is cloned from human leukocyte genomic DNA by PCR.

- 5 The control region is amplified by PCR using synthetic forward and reverse primers. The forward and reverse primers used for cloning human ELG4 control region by PCR amplification are 5'-CGACGCGTTGCGCCTGGCTGAACACTAC-3' and 5'-GGAAGATCTCTGGGACAAACAACAGGC-3', respectively. The *Mlu*I and *Bgl*II recognition sequences (underlined), respectively, are included to facilitate cloning.

10

The PCR amplification and cloning are performed as described in Example 9.

The resulting human ELG4 control region/reporter construct is used and to transfect different mammalian cell lines, and reporter activity measured.

15

**Example 13 - Cloning Human ELG5 Control Region**

The ELG5 control region (1411 bp) is cloned from human leukocyte genomic DNA by PCR. The control region is amplified by PCR using synthetic forward and reverse primers starting at positions -1411 bp and -1 bp upstream the translation initiation codon, ATG. The forward and reverse primers used for cloning human ELG5 control region by PCR amplification are 5'-CCGCTCGAGGTGAGCCACCACCGCGGCC-3' and 5'-CCGCTCGAGTGGGGCTGATCTTCGGAGTCGC-3', respectively. The *Xho*I recognition sequence (underlined) is included to facilitate cloning.

25

The PCR amplification and cloning are performed as described in Example 9.

The resulting human ELG5 control region/reporter construct is used to transfect different mammalian cell lines, and reporter activity measured.

30

**Example 14 - Cloning Human ELG6 Control Region**

The ELG6 control region (1937 bp) is cloned from human leukocyte genomic DNA by PCR. The control region is amplified by PCR using synthetic forward and reverse primers starting at positions -1937 bp and -1 bp upstream the initiation codon, ATG. The forward and reverse primers used for cloning human ELG6 control region by PCR amplification are 5'-

35

CCGAGCTCGATTAGCTGTCAGGCTATATATGGAGCC-3' and 5'-  
CCGAGCTCCTAGTTTGCAGAAGGTCCAAAGC-3', respectively. The *SacI* recognition  
sequence (underlined) is included to facilitate cloning.

- 5 The PCR amplification and cloning are performed as described in Example 9.

The resulting human ELG6 control region/reporter construct is used to transfect different  
mammalian cell lines, and reporter activity measured.

10 **Example 15 - Cloning Human ELG7 Control Region**

The ELG7 control region (2000 bp) is cloned from human leukocyte genomic DNA by PCR.  
The control region is amplified by PCR using synthetic forward and reverse primers starting at  
positions -2000 bp and -1 bp upstream the translation initiation codon, ATG. The forward and  
reverse primers used for cloning human ELG7 control region by PCR amplification are 5'-  
CCGAGCTCGGAAATACCTGAAGCTGTTTAAAC-3' and 5'-  
CCGAGCTCCGCGGCGATGAGCGGGC-3', respectively. The *SacI* recognition sequence  
(underlined) is included to facilitate cloning.

- 20 The PCR amplification and cloning are performed as described in Example 9.

The resulting human ELG7 control region/reporter construct is used to transfect different  
mammalian cell lines, and reporter activity measured.

25 **Example 16 - Drug Screening Assay Using ELG3 Control Region.**

Plasmid pGh3020.1 (Figure 20), containing the ELG3 control region, is used to screen test  
compounds that modulate the ELG3 promoter activity. Transient transfections are performed  
to evaluate the functionality of the ELG3 control region using techniques known by persons  
skilled in the art.

Alternatively, HepG2 cells are stably transfected with 10 µg of pGh3020.1 and 1 µg of vector  
pRSV-NEO (ATCC), using 10 µl of Lipofectamine 2000 Reagent (Gibco BRL) in a 60 mm  
tissue culture dish as described by the manufacturer. After a 24 h incubation, the cells are  
passaged into two 150 mm tissue culture dishes at a 1:2 dilution and grown for another 24 h.  
Geneticin (Gibco BRL) is added to the medium at a concentration of 800 µg/ml. After 3-4

weeks of growth under the selection pressure of the antibiotic, the resistant clones are isolated and characterized for their luciferase activity.

Drug screening is performed using the Luciferase Enzyme Assay System (Promega),  
5 following the manufacturer's recommendations. Briefly, transfected cells grown in a 96 well plate are exposed to test compounds. After an appropriate incubation time, the cells are washed with  $Mg^{2+}$  and  $Ca^{2+}$  free PBS. Cells are lysed with 20  $\mu$ l of 1X Luciferase Cell Culture Lysis Reagent (CCLR, Promega). The plate is placed into a luminometer with an automatic injector. For each well, the injector adds 100  $\mu$ l of Luciferase Assay Reagent (Promega), and  
10 the light emission generated by the reaction is read for 10 seconds after a 2 second delay. Cell cultures without a test compound are used as controls. Any significant difference in the luciferase activity indicates that the test compound is modulating the ELG3 promoter activity.

This assay or other reporter assays are suitable for drug screening using the control region of  
15 any elongase gene.

#### Example 17 - Drug Screening Assays Using Yeast One-Hybrid Systems

Methods for yeast one-hybrid assays are known by persons skilled in the art (Fields S. and  
20 Song O., 1989, *Nature*, 340: 245-246 and Ulmasov et al., 1997, *Science*, 276: 1865-1868). Reagents and/or kits are commercially available for the assays, e.g., the Matchmaker One-Hybrid System (Clontech).

This assay is suitable for all of the elongase control regions described herein.  
25

The known target elements, or elongase control region 'bait' is inserted upstream of a reporter gene (e.g. *HIS3*) and integrated into the yeast genome to make a new reporter strain. The yeast strain is transformed with an activation domain (AD) fusion library to screen for DNA binding proteins that interact with the bait DNA sequence. Binding of an AD/DNA-binding domain  
30 (DBD) hybrid protein to the target sequence results in activation of the reporter gene transcription and subsequent selection. For example, expression of *HIS3* will allow colony growth on minimal medium lacking histidine. The cDNA encoding DNA binding protein (DBP) is isolated and characterized. The interaction is reconstructed *in vitro* or *in vivo* for screening test compounds by exposing the target elements or elongase control region to the  
35 DBP in the presence of test compounds. The effect of the test compound is evaluated through assays, well known to those skilled in the art, that measure DNA/protein binding interactions.

**Example 18 - Drug Screening Assays Using Yeast Two-Hybrid Systems**

Methods for the yeast two-hybrid assays are known by persons skilled in the art (Fields S. and Song O., 1989, *Nature*, 340: 245-246 and Furuyama K. and Sassa S., 2000, *J. Clin. Invest.*, 105: 757-764). Reagents and/or kits are commercially available for the assays, e.g., the Hybrid Hunter Yeast Two-Hybrid (Invitrogen), the Matchmaker Two-Hybrid Systems (Clontech) and the HybriZAP Two Hybrid System (Stratagene).

10 This assay is suitable for all of the elongase genes disclosed herein.

Two physically distinct functional domains are necessary: a DNA binding domain (DBD) and an activation domain (AD). The elongase polypeptide of interest is cloned into a "bait" vector, and expressed as a hybrid protein with a DBD. A library of cDNAs encoding potential  
15 interacting proteins is cloned in frame with AD in the "prey" vector. The bait and prey vector fusion constructs are transformed into one of several-engineered yeast strains. If an interaction between bait and prey hybrid proteins occurs, the AD of the prey is brought into close contact with the DBD and transcription of the reporter genes is activated. Positive interacting proteins are easily identified by plating on nutrient deficient medium, and screening for reporter  
20 activity.

The interaction between these two proteins is reconstructed *in vitro* or *in vivo* for screening test compounds by exposing the two interacting proteins to test compounds. The effect of the test compound is evaluated through assays, well known to those skilled in the art, that measure  
25 protein/protein binding interactions.

**Example 19 - Functional Analysis of Human Elongases in *Saccharomyces cerevisiae***

The example presented herein demonstrates that the human elongase genes, ELG1, ELG2,  
30 ELG3, ELG4, ELG5, ELG6 and ELG7 cloned by the inventors, encode enzymes able to elongate, by at least two carbons, n-3 and/or n-6 fatty acid substrates.

**Materials**

Lithium [1-<sup>14</sup>C]18:3n-6, [1-<sup>14</sup>C]18:3n-3, [1-<sup>14</sup>C]20:4n-6, and [1-<sup>14</sup>C]20:5n-3 (99%  
35 radiochemical purity; specific activity: 48 to 58 µCi/µmol), were purchased from NEN (Boston, MA). All unsaturated fatty acids were saponified with 0.1 M LiOH and dissolved in a synthetic minimal medium lacking uracil (SC-U) with 1% tergitol.

Fatty acid free bovine serum albumin, tergitol, Tris-HCl, carbohydrates, amino acids and fatty acids were obtained from Sigma-Aldrich Canada (ON, Canada). Yeast nitrogen base without amino acids was purchased from Difco (Becton Dickinson). All organic solvents (HPLC grade) were obtained from Fisher-Scientific (Fair Lawn, NJ).

#### Yeast Transformation

*Saccharomyces cerevisiae* strain INVSc1 (Invitrogen) was transformed with the elongase constructs previously described (Examples 1-7) or pYES2/CT using the lithium acetate method as supplied by Invitrogen. For the expression of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 or ELG7 the yeast were transformed with pTh1009.1, pTh1014.1, pTh1015.1, pTh1021.1, pTh1018.1, pTh1041.1 or pTh1044.1, respectively. For the expression of ELG1/V5-His, ELG2/V5-His, ELG3/V5-His, ELG4/V5-His, ELG5/V5-His, ELG6/V5-His or ELG7/V5-His the yeast were transformed with pTh1009.2, pTh1014.2, pTh1017.1, pTh1021.2, pTh1019.1, pTh1042.1 or pTh1045.1, respectively. Recombinant yeast cells were selected on SC-U medium.

#### Incubation

Transformed yeast (approximately  $3.2 \times 10^6$  cells/ml; O.D.<sub>600</sub> 0.4) were incubated in a 125 ml Erlenmeyer containing 10 ml of SC-U medium with 1% raffinose, 1% tergitol and 25  $\mu$ M of the lithium salts of either [1-<sup>14</sup>C]18:3n-3 (1  $\mu$ Ci), [1-<sup>14</sup>C]18:3n-6 (1  $\mu$ Ci), [1-<sup>14</sup>C]20:4n-6 (2  $\mu$ Ci), or [1-<sup>14</sup>C]20:5n-3 (2  $\mu$ Ci). After 4 h incubation in an orbital incubator at 270 rpm and 30°C, cells reached the log phase and the transgene expression was induced with galactose (2% final concentration). The yeast were incubated for an additional 19 h and then harvested by centrifugation at 5000 x g for 10 minutes at 4°C.

Cells were washed with Tris-HCl buffer (100 mM, pH 8.0) containing 0.1% BSA and total lipids were extracted as described below. The radioactivity from aliquots of the incubation medium, supernatant and cells was determined by liquid scintillation counting using a LS6500-Scintillation System (Beckman).

The host yeast transformed with pYES2/CT was used as negative control.

#### Lipid Extraction

Total lipids were extracted from cells with chloroform/methanol (2:1 v/v) according to the method of Folch et al., 1957, *J. Biol. Chem.*, 226: 497-509. Alternatively, cells were

resuspended in 1.5 ml of water and saponified with 2 ml of 10% KOH in ethanol. The total lipid extracts or the free fatty acids from the saponified samples were methylated using boron trifluoride in methanol at 90°C for 30 min. The resultant methyl esters (FAME) were analyzed as described below.

5

#### Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) Analysis

Analyses of radiolabelled FAME were carried out on a Hewlett Packard 1090, series II chromatograph equipped with a diode array detector set at 205 nm, a radioisotope detector (model 171, Beckman, CA) with a solid scintillation cartridge (97% efficiency for  $^{14}\text{C}$ -  
10 detection) and a reverse-phase ODS (C-18) Beckman column (250 mm x 4.6 mm i.d.; 5  $\mu\text{m}$  particle size) attached to a pre-column with a  $\mu\text{Bondapak C-18}$  (Beckman) insert. FAME were separated isocratically with acetonitrile/water (95:5 v/v) at a flow rate of 1 ml/min and were identified by comparison with authentic standards. Alternatively, the eluted FAME were collected and the solvent evaporated. FAME were re-dissolved in hexane for further analysis  
15 by gas chromatography.

#### Gas Chromatography (GC) Analysis

The FAME profile was determined using a Hewlett Packard Gas Chromatograph equipped with an interfaced ChemStation, a flame-ionization detector and a 30 m x 0.25 mm i.d. fused  
20 silica column (HP-wax, cross linked polyethylene glycol, film thickness 0.25  $\mu\text{m}$ ) and He as gas carrier. The temperatures of the injector and detector were maintained at 225°C and 250°C, respectively. After an initial hold of 1 min at 180°C, the column temperature was increased by 4°C/min to 190°C (7 min hold), then by 10°C/min to 200°C (5 min hold) and finally by 25°C/min to 215°C. This temperature was maintained for 17.9 min. FAME were  
25 identified by comparison with authentic standards.

#### Results

RP-HPLC analyses revealed that the exogenously added radiolabelled polyunsaturated fatty acids were elongated by at least two carbons in yeast transformed with human elongase genes  
30 (Table 3). In yeast expressing ELG4, 18:3n-6 was converted into 20:3n-6 which was then elongated to 22:3n-6, 20:4n-6 was converted into 22:4n-6 which was further elongated to 24:4n-6 and 18:3n-3 was converted into 20:3n-3 and 22:3n-3 (Figure 21). Yeast transformed with pYES2/CT did not elongate any of these substrates (Figure 22).

35 In yeast expressing elongases with V5-His tag, the percent elongation of selected substrates was similar to that detected in yeast with non-tagged enzymes (Table 4).



Conclusion

The functional analysis of the human ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7 genes confirmed that each gene encodes a fatty acid elongase which is active on various

5 PUFAs.

Table 3

Percent Elongation of PUFA Substrates to their Products in Yeast Expressing Human

Elongases

10

Gene	Plasmid	18:3n-6		20:4n-6		18:3n-3		20:5n-3	
		20:3	22:3	22:4	24:4	20:3	22:3	22:5	24:5
ELG1	pTh1009.1	2	nd	6	2	1	nd	2	nd
ELG2	pTh1014.1	62	3	39	1	16	nd	59	nd
ELG3	pTh1015.1	10	nd	11	21	2	nd	16	29
ELG4	pTh1021.1	20	4	24	2	10	4	15	3
ELG5	pTh1018.1	3	nd	nd	nd	9	nd	-	-
ELG6	pTh1041.1	2	nd	nd	nd	3	nd	nd	nd
ELG7	pTh1044.1	nd	nd	nd	nd	5	nd	nd	nd

nd: not detected

-: not tested

Table 4

5     Percent Elongation of PUFA Substrates to their Products in Yeast Expressing V5-His Tagged  
Human Elongases

Gene	Plasmid	18:3n-6		20:4n-6		18:3n-3		20:5n-3	
		20:3	22:3	22:4	24:4	20:3	22:3	22:5	24:5
ELG1	pTh1009.2	-	-	7	nd	-	-	-	-
ELG2	pTh1014.2	73	11	-	-	-	-	-	-
ELG3	pTh1017.1	-	-	8	15	-	-	-	-
ELG4	pTh1021.2	-	-	12	nd	-	-	-	-
ELG5	pTh1019.1	5	-	-	-	-	-	-	-
ELG6	pTh1042.1	nd	nd	Nd	nd	3	nd	nd	nd
ELG7	pTh1045.1	nd	nd	Nd	nd	4	nd	nd	nd

10     nd: not detected

         -: not tested

Example 20 - Drug Screening Assay for Elongases Using Yeast

15     This example provides a methodology suitable for screening test compounds that modulate the activity of recombinant elongases in whole cells and spheroplasts of *Saccharomyces cerevisiae*. The test compound uptake is likely to be enhanced in yeast spheroplasts due to their lack of a cell wall. Thus, this is the model of choice for assessing the effect of low concentrations of test compounds on elongase activity.

20

Spheroplast Preparation

*Saccharomyces cerevisiae* heterologous for any of the human elongase genes are grown in SC-U medium with 1% raffinose and 2% galactose to induce the expression of the transgene.

After 16 h incubation, cells are centrifuged at 2060 x g for 5 min at 4°C, washed once with

25     distilled water and centrifuged again. The volume and weight of the cell pellet are measured.

Cells are suspended (1:2 w/v) in 0.1 M Tris.SO<sub>4</sub> (pH 9.4), 10 mM DTT and incubated at 30°C.

After 10 min incubation, the cell pellet is obtained by centrifugation, washed once (1:20 w/v) with 1.2 M sorbitol and suspended (1:1 w/v) in 1.2 M sorbitol, 20 mM phosphate buffer (pH 7.4) as described elsewhere (Daum et al., 1982, *J. Biol. Chem.*, 257: 13028-13033). A 15,800 x g (1 min) supernatant of lyticase is added to the cell suspension at a concentration of 2000 U/ml and the suspension incubated at 30°C with 50 rpm shaking. Conversion to spheroplasts is checked after 40 min incubation by diluting the suspension with distilled water followed by observation under the microscope (Schatz G. and Kovac L., 1974, *Meth. Enzymol.*, 31A: 627-632). After 70 min incubation, approximately 90% of the cells are converted to spheroplasts.

#### 10 Incubation of Spheroplasts with Test Compounds

Spheroplasts are harvested by centrifugation at 2060 x g for 5 min at 4°C and washed once with 1.2 M sorbitol. Spheroplasts are resuspended in SC-U medium with 1% raffinose, 1% tergitol, 1.2 M sorbitol and 2% galactose to maintain the induction conditions and to give an O.D.<sub>600</sub> reading of approximately 2.5-3.0. A 10 ml aliquot of the spheroplast suspension is transferred to a 125 ml Erlenmeyer flask and incubated with 200 µl of a test compound in ethanol (e.g. pebulate sulphoxide with a final concentration ranging from 0.01 to 100 µM) at 30°C in an orbital incubator at 270 rpm. After 30 min incubation, 1 µCi of a selected elongase substrate (i.e., lithium salts of [1-<sup>14</sup>C]18:3n-6, [1-<sup>14</sup>C]20:4n-6, [1-<sup>14</sup>C]20:5n-3 or [1-<sup>14</sup>C]18:3n-3) is added to the culture to a final concentration of 2 to 200 µM and further incubated for 120 min. Cell density is determined (O.D.<sub>600</sub>) and spheroplasts are harvested by centrifugation and washed with Tris-HCl buffer (100 mM, pH 8.0) containing 0.1% BSA. Total lipids are extracted and analyzed as described in Example 19.

#### Incubation of Whole Yeast with Test Compounds

25 *Saccharomyces cerevisiae* heterologous for any of the human elongase genes are incubated in a 125 ml Erlenmeyer flask containing 9 ml of SC-U medium with 1% raffinose, 1% tergitol (O.D.<sub>600</sub> 0.4, approximately 3.2 x 10<sup>6</sup> cells/ml) and 200 µl of a test compound in ethanol (e.g. pebulate sulphoxide, with a final concentration in the culture that range between 0.1 and 5 mM). After 1 h incubation in an orbital incubator at 270 rpm and 30°C, 1 µCi of a selected elongase substrate (i.e., lithium salts of [1-<sup>14</sup>C]18:3n-6, [1-<sup>14</sup>C]20:4n-6, [1-<sup>14</sup>C]20:5n-3 or [1-<sup>14</sup>C]18:3n-3) is added to the culture to a final concentration of 2 to 200 µM. After 4 h incubation with the inhibitor, cells reach the log phase and the transgene expression is induced with the addition of 1 ml of galactose to a final concentration of 2%. The yeast are incubated for an additional 19 h and then harvested by centrifugation at 5000 x g for 10 minutes at 4°C. Cells are washed with Tris-HCl buffer (100 mM, pH 8.0) containing 0.1% BSA and total lipids are extracted and analyzed as described in Example 19.

### Calculations

The elongase activity is determined by measuring the conversion of radiolabelled 18:3n-6 to 20:3n-6 and 22:3n-6, 20:4n-6 to 22:4n-6 and 24:4n-6, 18:3n-3 to 20:3n-3 and 22:3n-3 or 20:5n-3 to 22:5n-3 and 24:5n-3. The percent inhibition is calculated as described elsewhere (Kawashima et al., 1996, *Biosci. Biotech. Biochem.*, 60: 1672-1676):

$$\% \text{ Inhibition} = 100(\text{activity without the inhibitor} - \text{activity with the inhibitor}) / \text{activity without the inhibitor}$$

10

### Example 21 - Drug Screening Assay for Elongase Using Yeast Microsomes

This example teaches that microsomes from yeast with elongase transgenes contain all the enzymes required for testing the effect of test compounds on the activity of a specific recombinant fatty acid elongase.

15

### Materials

A sulfoxide derivative of S-propylbutylethylthiocarbamate (pebulate sulfoxide) was obtained from Zeneca Agrochemicals, UK, and dissolved in ethanol at a concentration of 5 mM.

20

### Yeast Microsome Preparation

A 5 l culture of *Saccharomyces cerevisiae* transformed with pTh1017.1 encoding ELG3/V5-His was started with a cell density of approximately  $3.2 \times 10^5$  cells/ml (O.D.<sub>600</sub> 0.4) using SC-U medium with 1% raffinose. After 8 h of incubation at 30°C in an orbital shaker at 270 rpm, galactose was added to a final concentration of 2%. Yeast were incubated for an additional 12 h until they were harvested by centrifugation at 2060 x g for 10 minutes at 4°C and washed with water. The cell pellet was resuspended in 1/3 of its volume in a pH 7.2 isolation buffer (80 mM Hepes-KOH, 10 mM KCl, 320 mM sucrose, 2 mM PMSF and a protease inhibitor cocktail). The cell suspension was poured into a mortar containing liquid N<sub>2</sub> and ground with sand using a ceramic pestle. The yeast powder was transferred to a conical test tube, to which 2/3 of the pellet volume of isolation buffer was added. The sand was removed by centrifugation at 228 x g for 1 min and the suspension centrifuged at 10,000 x g for 20 min to separate cell debris, nuclei and mitochondria. The supernatant was centrifuged at 106,000 x g for 1.5 h to obtain the microsomal pellet, which was resuspended in storage buffer (80 mM Hepes-KOH, 10 mM KCl, 320 mM sucrose, 1 mM PMSF and a protease inhibitor cocktail) to

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a final protein concentration of 20 µg/µl. The protein concentration was measured by the method of Lowry et al. (1951, *J. Biol. Chem.*, 193: 265-275) with bovine serum albumin as standard.

5 Incubation of Yeast Microsomes with Pebulate Sulphoxide

The activity of ELG3/V5-His was determined by measuring the conversion of [1-<sup>14</sup>C]20:5n-3 to [1-<sup>14</sup>C]22:5n-3 and [1-<sup>14</sup>C]24:5n-3. Reactions were started by adding 500 µg of yeast microsomal protein to pre-incubated tubes containing 0.20 µCi of the substrate fatty acid at a final concentration of 7.2 µM in 0.25 ml of 80 mM Hepes-KOH (pH 7.2) with 43 mM MgCl<sub>2</sub>, 1.0 mM ATP, 500 µM NADPH, 10 µM coenzyme A, 100 µM malonyl-CoA (as lithium salt) and pebulate sulphoxide at concentrations that ranged between 1 to 100 µM. The tubes were vortexed vigorously and after 30 min incubation at 37°C in a shaking water bath, the reactions were stopped by the addition of 2 ml of 10% (w/v) KOH in ethanol. Lipids in the incubation mixture were saponified at 80°C for 45 min under N<sub>2</sub>. The samples were then left in ice for 5 min before acidification with 750 µl of concentrated HCl. The fatty acids were extracted with hexane and esterified with BF<sub>3</sub> in methanol at 90°C for 30 min. The fatty acid methyl esters were analyzed by HPLC as described in Example 19.

Results

20 The enzyme activity was expressed in percent conversion of radiolabelled 20:5n-3 into its elongation products. Alternatively, it can be expressed in pmol of the fatty acids produced/mg microsomal protein/min.

Table 5 shows the effect of a thiocarbamate derivative (pebulate sulphoxide) on the ELG3/V5-His activity when 20:5n-3 was provided as substrate. Pebulate sulphoxide at 100 µM substantially reduced elongation, by approximately 27%. This effect was mainly due to a reduction in the synthesis of 22:5n-3 rather than in the production of its metabolite, 24:5n-3.

Table 5

Effect of Pebulate Sulphoxide on the Elongation of [1-<sup>14</sup>C]20:5n-3 in Microsomes of Yeast  
Expressing ELG3/V5-His.

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Pebulate sulphoxide [ $\mu$ M]	% conversion		
	22:5n-3	24:5n-3	Total
0	13.7	5.0	18.7
1	13.8	5.6	19.4
10	12.8	6.6	19.4
50	11.3	4.6	15.9
100	9.4	4.3	13.7

Values expressed are the average (dispersion  $\leq$  10%) of two determinations.

Example 22 - Isolation of Recombinant Elongases from Yeast.

- 10 This example provides a methodology for the isolation of recombinant elongase from yeast homogenate or microsomes. The purified enzyme is useful for drug screening or for antibody production.

Yeast Homogenate and Microsome Preparation

- 15 Yeast cell fractionation was performed as described in Example 21 using yeast expressing ELG3/V5-His.

Elongase Solubilization

- 20 Yeast cell homogenate or yeast microsomes were resuspended in solubilization buffer (80 mM HEPES-KOH pH 7.2, 10 mM KCl, 320 mM sucrose, 1 mM PMSF, protease inhibitor cocktail, and 0.5 M NaCl) at 1.3 or 4 mg/ml, respectively. Zwittergent 3-14, *n*-octyl-beta-glucopyranoside or *n*-octyl-beta-thioglucopyranoside (Calbiochem, CA) was added to a final concentration of 2%, with a detergent:protein ratio of 15:1. The mixture was incubated for 2 h at 4°C with stirring and then centrifuged at 106,000 x g for 1 h. The supernatant was removed and stored at -80°C until use. The pellet was resuspended in 1/4 volume of the supernatant using solubilization buffer. The efficiency of each detergent to solubilize the elongase was determined by Western blot analysis as described below.
- 25

#### SDS-PAGE and Western Blot Analysis

Supernant (60  $\mu$ l) or pellet suspension (20  $\mu$ l) was mixed with 15  $\mu$ l or 5  $\mu$ l of 5X sample loading buffer (1X concentration: 50 mM Tris-HCl pH 8.0, 2% SDS, 10 mM beta-mercaptoethanol, 0.1% bromophenol blue, 10% glycerol), respectively, and boiled at 100°C for 5 minutes. Molecular weight standards (Santa Cruz Biotechnology, CA), controls, 25  $\mu$ l of the supernatant, and 12.5  $\mu$ l of the pellet were loaded on 12% pre-cast SDS-polyacrylamide gels. After electrophoresis, the protein was electro-transferred onto a PVDF membrane (Bio-Rad). The membrane was incubated with a blocking solution and subsequently probed with an anti-V5-HRP antibody as recommended by the manufacturer (Invitrogen). The membrane was washed and the antibody was detected using the enhanced chemiluminescence reagent, ECL (Amersham-Pharmacia Biotech.). The membrane was exposed to autoradiography film (Labscientific, NJ).

Zwittergent 3-14 was the most effective detergent in solubilizing ELG3/V5-His, the majority of the tagged protein having been detected in the 106,000 x g supernatant.

#### Immobilized Metal Ion Affinity Chromatography (IMAC)

The supernatant containing the solubilized enzyme is loaded onto a pre-equilibrated HiTrap chelating ( $\text{Ni}^{2+}$  charged iminodiacetate) column (Pharmacia) attached to a fast protein liquid chromatography system (Pharmacia). The column is washed with 50 mM sodium phosphate pH 8.0. The tagged protein is eluted with the same buffer containing imidazole ranging from 0 to 500 mM and further concentrated by ultrafiltration using Centriprep (Amicon) concentrators.

Alternatively, Macro-Prep ceramic hydroxyapatite (Bio-Rad, CA), TALON metal affinity resin (a Cobalt-based IMAC resin, Clontech, CA), Ni-nitriloacetic acid resin (Novagen, WI) or other similar resin is used.

#### Example 23 - Drug Screening Assay for Elongase Using Purified Enzyme

The concentrated enzyme (Example 22) is incubated at 30-37°C in 0.25 ml of 80 mM Hepes-KOH (pH 7.2) with 6 mM egg phosphatidylcholine, 2% Triton X-100, 0.4% sodium deoxycholate, 43 mM  $\text{MgCl}_2$ , 1.0 mM ATP, 500  $\mu$ M NADPH, 10  $\mu$ M coenzyme A, 100  $\mu$ M malonyl-CoA (as lithium salt), 0.20  $\mu$ Ci of the substrate fatty acid (i.e., radiolabelled eicosapentaenoyl-CoA) at a final concentration of 7.2  $\mu$ M and a test compound (e.g., pebulate

sulphoxide) at concentrations ranging between 0.01 to 100  $\mu$ M. The tubes are vortexed vigorously and after 30 min incubation at 37°C in a shaking water bath the reactions are stopped by the addition of 2 ml of 10% (w/v) KOH in ethanol.

Total lipids are extracted and methyl ester analyzed as described in Example 19.

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**Example 24 - Validation of Drug Screening Assays Described in Examples 20, 21 and 23 Using Rat Liver Microsomes**

**Preparation of Rat Liver Microsomes**

- 10 Wistar rats under light halothane (15% in mineral oil) anesthesia were sacrificed by exsanguination during periods of high enzyme activity. Livers were immediately rinsed with cold 0.9% NaCl solution, weighed and minced with scissors. All procedures were performed at 4°C unless specified otherwise. Livers were homogenized in a solution (1:3 w/v) containing 0.25 M sucrose, 62 mM potassium phosphate buffer (pH 7.0), 0.15 M KCl, 1.5 mM
- 15 *N*-acetylcysteine, 5 mM  $MgCl_2$ , and 0.1 mM EDTA using 4 strokes of a Potter-Elvehjem tissue homogenizer. The homogenate was centrifuged at 10,400 x g for 20 min to pellet mitochondria and cellular debris. The supernatant was filtered through a 3-layer cheesecloth and centrifuged at 105,000 x g for 60 min. The microsomal pellet was gently resuspended in the same homogenization solution with a small glass/teflon homogenizer and stored at -80°C.
- 20 The absence of mitochondrial contamination was enzymatically assessed as described elsewhere (Kilberg, M.S. and Christensen H.N., 1979, *Biochemistry*, 18: 1525-1530). The protein concentration was measured by the method of Lowry et al (1951, *J. Biol. Chem.*, 193: 265-275) with bovine serum albumin as standard.

25 **Incubation of Rat Liver Microsomes with Test Compounds**

Reactions were performed using 500  $\mu$ g of rat liver microsomal protein with the same concentrations of pebulate sulphoxide, radiolabelled fatty acid, conditions and procedures described in Example 21.

30 **Results**

The enzyme activity was expressed in percent conversion of radiolabelled 20:5n-3 into its elongation and final delta-6-desaturation products (i.e., 22:5n-3, 24:5n-3 and 24:6n-3). When the incubation was performed under nitrogen, the desaturation reaction did not occur.

- 35 Table 6 shows the effect of a thiocarbamate derivative (pebulate sulphoxide ) on the rat liver elongase activity when 20:5n-3 was provided as substrate. Pebulate sulphoxide (100  $\mu$ M)



reduced elongation by approximately 30%. This effect was mainly due to a reduction in the synthesis of 24:5n-3 rather than in the synthesis of 22:5n-3.

**Table 6**

**5      Effect of Pebulate Sulphoxide on the Elongation of [1-<sup>14</sup>C]20:5n-3 in Rat Liver Microsomes**

Pebulate sulphoxide [μM]	% conversion			
	22:5n-3	24:5n-3	24:6n-3*	Total
0	11.6	39.7	9.1	60.4
1	12.5	47.5	9.6	69.3
10	12.5	47.2	10.9	70.7
50	12.2	48.7	7.9	68.8
100	10.2	28.0	4.5	42.7

Values are expressed as the mean (dispersion ≤ 10%) of two determinations.

\* 24:6n-3 is the product of a delta-6-desaturation of 24:5n-3.

- 10      Since the rat liver microsomal and the recombinant human elongase (Example 21) activities were similarly affected by pebulate sulphoxide, it is concluded that rat liver microsomes are suitable to use in the validation of drug screening assays.

**15      Example 25 - Functional Characterization of Recombinant Fatty Acid Elongase and Desaturase in Yeast Co-expressing ELG3 and D6D**

This example shows a partial reconstitution of the n-3 and n-6 polyunsaturated fatty acid biosynthetic pathway in a heterologous host such as *Saccharomyces cerevisiae* using human fatty acid elongase and desaturase genes .

20

**Materials**

- 25      [1-<sup>14</sup>C]18:3n-3, [1-<sup>14</sup>C]20:4n-6, [1-<sup>14</sup>C]20:5n-3 and [1-<sup>14</sup>C]18:2n-6 (99% radiochemical purity; specific activity: 51 to 56 μCi/μmol) were purchased from NEN (Boston, MA). Fatty acids were saponified with 0.1 M LiOH and dissolved in synthetic minimal medium lacking either leucine (SC-Leu) or uracil and leucine (SC-U-Leu), containing 1% tergitol.

### Yeast Transformation

*Saccharomyces cerevisiae* strain INVSc1 (Invitrogen) was transformed using the lithium acetate method as supplied by Invitrogen. The coding sequence for human delta-6-desaturase (GenBank Accession No. AF126799) was previously cloned into the pYES2/CT vector for the production of the protein with a C-terminal tag containing the V-5 epitope and polyhistidine peptide (D6D/V5-His) as described in Canadian Patent Application No. 2,301,158, Mar., 2000, Winther et al. (plasmid designated pTh5002.1). For the co-expression of ELG3 and D6D/V5-His, the yeast were initially transformed with pTh5002.1. Recombinant yeast cells were selected on SC-U medium and then transformed with pLh5015.1 (Example 3). Double recombinant yeast cells containing both pTh5002.1 and pLh5015.1 were selected on SC-U-Leu medium. Yeast cells transformed with pBEVY-L alone, the cloning vector for ELG3, were selected on SC-Leu medium.

### Incubation

Transformed yeast cultures (approximately  $3.2 \times 10^6$  cells/ml; O.D.<sub>600</sub> 0.4) were divided in two experimental groups. The first group was incubated in a 125 ml Erlenmeyer flask containing 10 ml of SC-U-Leu medium with 2% raffinose, 1% tergitol and 25  $\mu$ M lithium [1-<sup>14</sup>C]20:4n-6 (1  $\mu$ Ci). Yeast of the second group were incubated in 10 ml of SC-U-Leu medium containing 1% raffinose, 2% galactose (to induce the expression of D6D/V5-His) and 1% tergitol. Lithium salts (1  $\mu$ Ci) of either [1-<sup>14</sup>C]18:3n-3, [1-<sup>14</sup>C]20:4n-6, [1-<sup>14</sup>C]20:5n-3 or [1-<sup>14</sup>C]18:2n-6 were added to both experimental groups at a final concentration of 25  $\mu$ M. After 24 h incubation in an orbital incubator at 270 rpm and 30°C, cells were harvested by centrifugation at 5000 x g for 10 minutes at 4°C.

The cell pellet was washed with Tris-HCl buffer (100 mM, pH 8.0) containing 0.1% BSA total lipids were extracted and radiolabelled fatty acids analyzed as described in Example 19.

The host yeast transformed with pBEVY-L was used as negative control.

### Results

Figures 23 and 24 show that only elongation products of PUFA substrates for ELG3 were detected when galactose was absent from the culture medium since the expression of D6D/V5-His was not induced. The constitutively expressed ELG3 was able to elongate 20:4n-6 to 22:4n-6 and 24:4n-6, 20:5n-3 to 22:5n-3 and 24:5n-3, and to a lesser extent 18:3n-3 to 20:3n-

3. These findings are consistent with those described in Example 19. ELG3 did not elongate 18:2n-6.

5 The elongation products of PUFA substrates for ELG3 were desaturated by D6D/V5-His when galactose was added to the medium (Figure 24). In this regard, 24:5n-6 and 24:6n-3 were produced from 24:4n-6 and 24:5n-3, respectively.

10 In the presence of galactose, transformed yeast were also able to delta-6-desaturate 18:2n-6 and 18:3n-3 to 18:3n-6 and 18:4n-3, respectively. These products were then substrates of the ELG3, which elongated them to 20:3n-6 and 20:4n-3, respectively.

Both ELG3 and D6D/V5-His seemed to be more active on n-3 than on n-6 fatty acid substrates.

15 Yeast transgenic for the human elongase, ELG3, and a human D6D, were able to generate polyunsaturated fatty acids of the so called "Sprecher pathway" (Sprecher H., 2000, *Biochim. Biophys. Acta*, 1486: 219-231). The present inventors are the first to report that products of human ELG3, 24:4n-6 and 24:5n-3, are substrates of a human D6D, which is also active on 18:2n-6 and 18:3n-3.

20

**Example 26 - Functional Characterization of Recombinant Fatty Acid Elongase and Desaturase in Yeast Co-expressing ELG3 and D5D**

25 This example expands the inventors' findings described in Example 25. The sequential elongation and desaturation of n-3 and n-6 PUFAs in a heterologous host co-expressing human fatty acid elongase and D5D genes is demonstrated.

**Materials**

30 [1-<sup>14</sup>C]18:3n-3, [1-<sup>14</sup>C]20:3n-6 and [1-<sup>14</sup>C]18:2n-6 (99% radiochemical purity; specific activity: 50 to 52 μCi/μmol) were purchased from NEN (Boston, MA). [1-<sup>14</sup>C]-Δ<sup>8,11,14,17</sup> eicosatetraenoic acid, 20:4n-3, (99% radiochemical purity; specific activity: 55 μCi/μmol) was purchased from ARC (St Louis, MO). Fatty acids were saponified with 0.1 M LiOH and dissolved in either SC-Leu or SC-U-Leu medium, containing 1% tergitol.

### Yeast Transformation

*Saccharomyces cerevisiae* strain INVSc1 (Invitrogen) was transformed using the lithium acetate method as supplied by Invitrogen. The coding sequence for human delta-5-desaturase (GenBank Accession No. AF199596) was previously cloned into the pYES2/CT vector for the production of the protein with a C-terminal tag containing the V-5 epitope and polyhistidine peptide (D5D/V5-His) as described in Canadian Patent Application No. 2,301,158, Mar., 2000, Winther et al. (plasmid designated pTh5009.1). For the co-expression of ELG3 and D5D/V5-His, the yeast were initially transformed with pTh5009.1. Recombinant yeast cells were selected on SC-U medium and then transformed with pLh5015.1 (described in Example 3). Double recombinant yeast cells containing both pTh5009.1 and pLh5015.1 were selected on SC-U-Leu medium. Yeast cells transformed with pBEVY-L alone, the cloning vector for ELG3, were selected on SC-Leu medium.

### Incubation

Cultures of transformed yeast (approximately  $3.2 \times 10^6$  cells/ml; O.D.<sub>600</sub> 0.4) were divided in two experimental groups. In the first group, cells were incubated in a 125 ml Erlenmeyer flask containing 10 ml of SC-U-Leu medium with 2% raffinose and 1% tergitol. In the second group, yeast were incubated in 10 ml of SC-U-Leu medium with 1% raffinose, 2% galactose (to induce the expression of D5D/V5-His) and 1% tergitol. Lithium salts (1  $\mu$ Ci) of either [ $1\text{-}^{14}\text{C}$ ]18:3n-3, [ $1\text{-}^{14}\text{C}$ ]20:3n-6, [ $1\text{-}^{14}\text{C}$ ]18:2n-6, or [ $1\text{-}^{14}\text{C}$ ]20:4n-3 were added to both experimental groups at a final concentration of 25  $\mu$ M. After 24 h incubation in an orbital incubator at 270 rpm and 30°C, cells were harvested by centrifugation at 5000 x g for 10 minutes at 4°C.

The cell pellet was washed with Tris-HCl buffer (100 mM, pH 8.0) containing 0.1% BSA, total lipids were extracted and radiolabelled fatty acids were analyzed as described in Example 19.

The host yeast transformed with pBEVY-L was used as negative control.

### Results

Figure 25 shows that 20:3n-6 was desaturated to 20:4n-6, which was further elongated to 22:4n-6 and 24:4n-6, when the yeast co-expressed both genes in the presence of galactose. When galactose was not added to the medium, 20:3n-6 was only elongated to 22:3n-6.

Similarly, D5D/V5-His desaturated 20:4n-3 producing 20:5n-3, which was then elongated to 22:5n-3 and 24:5n-3. The elongation of 20:4n-3 to 22:4n-3 and 24:4n-3 was also detected.

5 Under these experimental conditions, yeast co-expressing both genes was not able to elongate and further desaturate 18:2n-6. D5D/V5-His was not active on 20:3n-3, the direct elongation product of 18:3n-3 generated by ELG3 (Figure 26).

#### Conclusion

10 Yeast co-expressing ELG3 and a human D5D, both cloned by the inventors, were able to generate substrates (i.e., 24:4n-6 and 24:5n-3) of the so called "Sprecher pathway" (Sprecher H., 2000, *Biochim. Biophys. Acta*, 1486: 219-231).

#### Example 27 - Drug Screening Assays Using Whole Cells, Spheroplasts or Microsomes of Yeast Co-Expressing ELG3 and either Human D6D or D5D

15 The following assays are designed to identify compounds that affect the human elongase ELG3 and/or the human desaturases using one host system or any part thereof.

#### Spheroplast and Microsome Preparation

20 Transformed *Saccharomyces cerevisiae* cells are grown in SC-U-Leu medium with 1% raffinose and 2% galactose to induce the expression of the desaturase transgenes. After 16 h incubation, spheroplasts are obtained as described in Example 20.

25 Microsomes from host cells expressing both elongase and desaturase genes are prepared using the liquid N<sub>2</sub> and differential centrifugation methods described in Example 21.

#### Incubation of Whole Yeast Cells, Spheroplasts or Microsomes with Test Compounds

30 In these assays with yeast cells containing elongase and desaturase transgenes, the use of SC-U-Leu medium is required to maintain selection pressure. Transformed yeast are incubated with or without galactose to assess the effect of the test component on the activity of ELG3 and the desaturases or the elongase alone, respectively. The substrates of choice are 20:3n-6 or 20:5n-6 for yeast expressing ELG3 and D5D or ELG3 and D6D, respectively. The incubation conditions of whole yeast cells, spheroplasts or microsomes with test compounds are the same as those described in Examples 20 and 21. Regardless of the host system used, the effect of the  
35 test compound on the activity of the recombinant enzymes is determined by the RP-HPLC or GC analysis of the relative amounts of FAME produced by ELG3 and/or the desaturases as described in Example 19.

**Example 28 - Elongation of PUFAs in Primary Cultures of Leukocytes from Control and STZ-Induced Diabetic Rats**

- 5 The present example describes the capability of leukocytes to elongate but not desaturate PUFAs. The example also provides details of how the elongation of 18:3n-6 and 18:2n-6 is affected in rats with STZ-induced diabetes.

**Materials**

- 10 RPMI 1640 medium was obtained from Gibco BRL. Streptozotocin (2-desoxy-2-methylnitrosoamino carbonyl amino-D-glucopyranose) was supplied by Sigma.

**Animals**

- Female Wistar rats were obtained from Charles Rivers, St-Constant, Quebec. Animals were  
15 housed in barrier-maintained rooms at 22±2°C, a target relative humidity of 50±10% with 15 air changes per hour and a 12 h light/dark cycle. Water and regular chow were provided *ad libitum*.

- All animals were monitored daily according to standard procedures in compliance with the Canadian Council of Animal Care guidelines for animal experimentation. Fifteen randomly  
20 selected rats were intraperitoneally (I.P.) injected with 50 mg of STZ per kg of body weight. Nine days later, animals received a second dose of STZ (75 mg/kg body weight). A second group of 12 rats which were sham injected with sterile 0.9% NaCl served as control. Two and 7 weeks after the last I.P. injection, control and STZ-treated rats (blood glucose levels 21 to >33 mmol/L) were put under light halothane (15% in mineral oil) anesthesia and sacrificed by exsanguination. Blood  
25 was collected into a 10 ml syringe containing 200 µl of a 5% solution of EDTA as anticoagulant.

**Leukocyte Isolation**

- Leukocytes were obtained by mixing 1 volume of whole blood with 5 volumes of sterile erythrocyte lysis buffer (Qiagen, CA). The cell suspension was incubated for 20 min on ice  
30 and centrifuged at 400 x g for 10 min at 4°C. The supernatant was discarded and the leukocyte pellet was washed and resuspended in 550 µl of 0.9% saline. Aliquots were taken for cell counting. Cellular protein content was measured using the method of Lowry et al (1951, *J. Biol. Chem.*, 193: 265-275) with bovine serum albumin as standard.

### Incubation

The present inventors' preliminary studies carried out with leukocytes isolated from Wistar rats showed that leukocytes can elongate 18:2n-6, 18:3n-3, 18:3n-6, 20:3n-6 and 20:4n-6 with the elongation of 18:2n-6 and 18:3n-6 being 6% and 66%, respectively, within 24 h. Based on these results and due to the impairment of D6D in diabetes, 18:2n-6 and 18:3n-6, substrate and product of D6D, respectively, were selected for the incubation of leukocytes from control and STZ-induced diabetic rats. No delta-6-desaturation on 18:2n-6, 18:3n-3 or delta-5-desaturation on 20:3n-6, was detected.

Leukocytes from the 2 and 7 week control group, as well as from the 2 and 7 week STZ-treated rat group, were incubated in RPMI 1640 medium with glutamine, 10% fetal calf serum and antibiotics (50 IU/ml penicillin, 50 µg/ml streptomycin) with 5 µM [1-<sup>14</sup>C]18:3n-6 (0.6 µCi) for 10 min to 24 h or with 5 µM of [1-<sup>14</sup>C]18:2n-6 (0.6 µCi) for 24 h.

At the end of each incubation, the cell pellet was obtained by centrifugation at 400 x g for 10 min at 4°C. Cells were washed with PBS containing 0.1% bovine serum albumin. Total cellular lipids were extracted with chloroform:methanol (2:1 v/v). Fatty acids were methylated with BF<sub>3</sub> and analyzed by RP-HPLC as described in Example 19. Alternatively, FAME can be analyzed by GC as described in Example 19.

### Results

Table 7 shows that leukocytes from STZ-induced diabetic rats rapidly converted 18:3n-6 into 20:3n-6. There was a significant increase in the activity of the elongation system in the STZ group, regardless of the time after the last I.P. STZ injection. Conversely, there was an approximately 50% reduction in the elongation of 18:2n-6 to 20:2n-6 in leukocytes obtained 2 weeks after the STZ injection (Table 8). There were no significant changes in the elongation of 18:2n-6 to 20:2n-6 in leukocytes from animals sacrificed 7 weeks after the STZ treatment.

Table 7

Conversion of 18:3n-6 into 20:3n-6 in Leukocytes from STZ-Induced Diabetic Rats Sacrificed  
2 or 7 Weeks Post-Induction

5

Incubation time (h)	2 weeks		7 weeks	
	STZ	Control	STZ	Control
0	0	0	0	0
0.16	50 ± 8	31 ± 9	37 ± 9	33 ± 4
0.5	115 ± 26	70 ± 12	112 ± 10	71 ± 15
1	288 ± 23	200 ± 16	190 ± 92	143 ± 31
24	nt	nt	1008 ± 98	628 ± 156

Values are expressed in pmol of 20:3n-6 produced/mg cellular protein and represent the mean ± S.D. of 6 rats.

10 nt: not tested

Table 8

15 Conversion of 18:2n-6 into 20:2n-6 in Leukocytes from STZ-Induced Diabetic Rats Sacrificed  
2 or 7 Weeks Post-Induction

2 weeks		7 weeks	
STZ	Control	STZ	Control
322 ± 119	126 ± 27	147 ± 22	128 ± 32

20 Leukocytes were incubated for 24 h.

Values are expressed in pmol of 20:2n-6 produced/mg cellular protein and represent the mean ± S.D. of 6 rats.

25 PUFA metabolism is altered in leukocytes of rats with STZ-induced diabetes. Therefore, leukocytes are an appropriate model to assess the modification or regulation of the elongation system in disease (e.g., diabetes).



**Example 29 - Elongation of PUFAs in Primary Cultures of Leukocytes from Humans**

This example shows that human leukocytes are a suitable model to assess elongase activity on 18:3n-6. This assay may be used in clinical trials to determine alterations in the elongation system in diseases such as diabetes.

Peripheral venous blood from fasted healthy volunteers (30 to 50 years of age) was obtained using 10 ml Vacutainers (Vacutainer Systems, NJ) containing EDTA as anticoagulant.

Leukocytes were isolated using the techniques described in Example 28. The incubation of leukocytes with 5  $\mu\text{M}$  [ $1\text{-}^{14}\text{C}$ ]18:3n-6 (0.6  $\mu\text{Ci}$ ) for 10 to 60 min was performed under the same conditions described in Example 28.

**Results**

Table 9 demonstrates that human leukocytes have a capability to rapidly elongate 18:3n-6 to 20:3n-6, similar to that found in rat leukocytes (Example 28). No delta-5-desaturation activity was detected on 20:3n-6.

**Table 9****Conversion of 18:3n-6 into 20:3n-6 in Leukocytes from Male and Female Volunteers**

Incubation time (h)	Male	Female
0	0	0
0.16	24 $\pm$ 5	25 $\pm$ 4
1	142 $\pm$ 60	157 $\pm$ 50
24	1479 $\pm$ 249	2233 $\pm$ 778

Values are expressed in pmol of 20:3n-6 produced/mg cellular protein and represent the mean  $\pm$  S.D. of 4 volunteers.

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## CLAIMS

We claim:

1. An isolated polynucleotide sequence, comprising a polynucleotide sequence which is  
5 selected from the group consisting of:
  - (a) a sequence comprising SEQ ID NO: 4;
  - (b) a sequence comprising SEQ ID NO: 8;
  - (c) a sequence comprising SEQ ID NO: 11;
  - (d) a sequence which is at least 80% homologous with a sequence of any of (a) to (c);
  - 10 (e) a sequence which is at least 90% homologous with a sequence of any of (a) to (c);
  - (f) a sequence which is at least 95% homologous with a sequence of any of (a) to (c);
  - (g) a sequence which is at least 98% homologous with a sequence of any of (a) to (c);
  - (h) a sequence which is at least 99% homologous with a sequence of any of (a) to (c);
  - and;
  - 15 (i) a sequence which hybridizes to any of (a) to (h) under stringent conditions.
2. An isolated polynucleotide sequence of claim 1, wherein the isolated polynucleotide sequence is cDNA.
- 20 3. A vector comprising a polynucleotide sequence of claim 1 in a suitable vector.
4. A host cell comprising a polynucleotide sequence of claim 1 in a host cell which is heterologous to said sequence.
- 25 5. An isolated polynucleotide fragment selected from the group consisting of:
  - (a) a sequence having at least 15 sequential bases of nucleotides of a sequence of claim 1;
  - (b) a sequence having at least 30 sequential bases of nucleotides of a sequence of claim 1; and
  - (c) a sequence having at least 50 sequential bases of nucleotides of a sequence of claim 1.
- 30 6. A vector comprising a polynucleotide sequence of claim 5 contained in a vector which is heterologous to said sequence.
7. A vector of claims 3 or 6, wherein said vector contains or encodes a tag.

8. An isolated polynucleotide sequence, comprising a polynucleotide sequence which retains substantially the same biological function or activity as or is a functional derivative of a polynucleotide of claim 1.
- 5 9. A method for identifying a compound which inhibits or promotes the activity of a polynucleotide sequence of claim 1, comprising the steps of:
- (a) selecting a control animal having said sequence and a test animal having said sequence;
- (b) treating said test animal using a compound; and,
- 10 (c) determining the relative quantity of an expression product of said sequence, as between said control animal and said test animal.
10. A method of claim 9, wherein said animals are mammals.
11. A method of claim 10, wherein said mammals are rats.
- 15 12. A method for identifying a compound which inhibits or promotes the activity of a polynucleotide sequence of claim 1, comprising the steps of:
- (a) selecting a host cell of claim 4;
- (b) cloning said host cell and separating said clones into a test group and a control group;
- 20 (c) treating said test group using a compound; and
- (d) determining the relative quantity of an expression product of said sequence, as between said test group and said control group.
13. A method for identifying a compound which inhibits or promotes the activity of a polynucleotide sequence of claim 1, comprising the steps of:
- 25 (a) selecting a test group having a host cell of claim 4 or a part thereof, and selecting a suitable control group;
- (b) treating said test group using a compound; and
- (c) determining the relative quantity or relative activity of a product of said sequence or of the
- 30 said sequence, as between said test group and said control group.
14. An isolated polypeptide comprising an isolated polypeptide selected from the group consisting of:
- (a) a sequence comprising SEQ ID NO: 5;
- 35 (b) a sequence comprising SEQ ID NO: 9;
- (c) a sequence comprising SEQ ID NO: 12;
- (d) a sequence which is at least 80% homologous with a sequence of any of (a) to (c);

- (e) a sequence which is at least 90% homologous with a sequence of any of (a) to (c);  
(f) a sequence which is at least 95% homologous with a sequence of any of (a) to (c);  
(g) a sequence which is at least 98% homologous with a sequence of any of (a) to (c);  
and  
5 (h) a sequence which is at least 99% homologous with a sequence of any of (a) to (c).
15. A host cell comprising a polypeptide sequence of claim 14 in a host cell which is heterologous to said sequence.
- 10 16. A process for producing a polypeptide sequence of claim 14 comprising the step of culturing the host cell of claim 15 under conditions sufficient for the production of said polypeptide.
- 15 17. An isolated polypeptide sequence, comprising a polypeptide sequence which retains substantially the same biological function or activity as a polypeptide of claim 14.
18. A method for identifying a compound which inhibits or promotes the activity of a polypeptide sequence of claim 14, comprising the steps of:  
(a) selecting a control animal having said sequence and a test animal having said sequence;  
20 (b) treating said test animal using a compound;  
(c) determining the relative quantity or relative activity of an expression product of said sequence or of the said sequence, as between said control animal and said test animal.
19. A method of claim 18, wherein said animals are mammals.
- 25 20. A method of claim 19, wherein said mammals are rats.
21. A method for identifying a compound which inhibits or promotes the activity of a polypeptide sequence of claim 14, comprising the steps of:  
30 (a) selecting a host cell of claim 15;  
(b) cloning said host cell and separating said clones into a test group and a control group;  
(c) treating said test group using a compound; and  
(d) determining the relative quantity or relative activity of an expression product of said sequence or of the said sequence, as between said test group and said control group.
- 35 22. A method for identifying a compound which inhibits or promotes the activity of a polypeptide sequence of claim 14, comprising the steps of:



- (a) selecting a test group having a host cell of claim 15 or a part thereof, and selecting a suitable control group;
- (b) treating said test group using a compound; and
- (c) determining the relative quantity or relative activity of a product of said sequence or of the said sequence, as between said test group and said control group.

23. An isolated polynucleotide sequence, comprising a polynucleotide sequence which is selected from the group consisting of:
- (a) a sequence comprising SEQ ID NO: 1;
- 10 (b) a sequence comprising SEQ ID NO: 2;
- (c) a sequence comprising SEQ ID NO: 3;
- (d) a sequence comprising SEQ ID NO: 6;
- (e) a sequence comprising SEQ ID NO: 7;
- (f) a sequence comprising SEQ ID NO: 10;
- 15 (g) a sequence comprising SEQ ID NO: 13;
- (h) a sequence which is at least 80% homologous with a sequence of any of (a) to (g);
- (i) a sequence which is at least 90% homologous with a sequence of any of (a) to (g);
- (j) a sequence which is at least 95% homologous with a sequence of any of (a) to (g);
- (k) a sequence which is at least 98% homologous with a sequence of any of (a) to (g);
- 20 (l) a sequence which is at least 99% homologous with a sequence of any of (a) to (g);
- and;
- (m) a sequence which hybridizes to any of (a) to (l) under stringent conditions.

24. An isolated polynucleotide sequence of claim 23, wherein the isolated polynucleotide sequence is genomic DNA.

25. A vector comprising a polynucleotide sequence of claim 23 in a suitable vector.

26. A host cell comprising a polynucleotide sequence of claim 23 in a host cell which is heterologous to said sequence.

27. A process for producing a polypeptide encoded by a gene operably linked to a polynucleotide sequence of claim 23 comprising the step of culturing the host cell of claim 26 under conditions sufficient for the production of said polypeptide.

28. An isolated polynucleotide fragment selected from the group consisting of:
- (a) a sequence having at least 15 sequential bases of nucleotides of a sequence of claim 23;

- (b) a sequence having at least 30 sequential bases of nucleotides of a sequence of claim 23;  
and
- (c) a sequence having at least 50 sequential bases of nucleotides of a sequence of claim 23.

5     29.     A vector comprising a polynucleotide sequence of claim 28 contained in a vector which is heterologous to said sequence.

10     30.     An isolated polynucleotide sequence, comprising a polynucleotide sequence which has substantially the same biological function or activity or is a functional derivative of a sequence of claim 23.

15     31.     A method for identifying a compound which inhibits or promotes the activity of a polynucleotide sequence of claim 23, comprising the steps of:  
      (a) selecting a control animal having said sequence and a test animal having said sequence;  
      (b) treating said test animal using a compound; and,  
      (c) determining the relative quantity of an expression product of an operably linked polynucleotide to said sequence, as between said control animal and said test animal.

20     32.     A method of claim 31, wherein said animals are mammals.

33.     A method of claim 32, wherein said mammals are rats.

25     34.     A method for identifying a compound which inhibits or promotes the activity of a polynucleotide sequence of claim 23, comprising the steps of:

- (a) selecting a host cell of claim 26;
- (b) cloning said host cell and separating said clones into a test group and a control group;
- (c) treating said test group using a compound; and
- (d) determining the relative quantity of an expression product of an operably linked polynucleotide to said sequence, as between said test group and said control group.

30     35.     A method for identifying a compound which inhibits or promotes the activity of a polynucleotide sequence of claim 23, comprising the steps of:

- (a) selecting a test group having a host cell of claim 26 or a part thereof, and selecting a suitable control group;
- 35     (b) treating said test group using a compound; and
- (c) determining the relative quantity of an expression product of an operably linked polynucleotide to said sequence, as between said test group and said control group.

36. A composition for treating a PUFA disorder comprising a compound which modulates a sequence according to claims 1, 14 or 23 and a pharmaceutically acceptable carrier.
- 5 37. A composition as claimed in claim 36, wherein said disorder is selected from the group consisting eczema, cardiovascular, inflammation, Sjögren's syndrome, gastrointestinal disorders, viral diseases and postviral fatigue, body weight disorders, psychiatric disorders, cancer, cystic fibrosis, endometriosis, pre-menstrual syndrome, alcoholism, congenital liver disease, Alzheimer's syndrome, hypercholesterolemia, autoimmune disorders, atopic disorders, acute respiratory distress syndrome, articular cartilage degradation, diabetes and diabetic complications.
- 10 38. A composition as claimed in claim 37, wherein said compound is selected from the group consisting of small organic molecules, peptides, polypeptides, antisense molecules, oligonucleotides, polynucleotides, fatty acids and derivatives thereof.
- 15 39. The use of a composition as claimed in claim 36 for treating PUFA disorders.
40. The use of claim 41, wherein said disorder is selected from the group consisting of eczema, cardiovascular, inflammation, Sjögren's syndrome, gastrointestinal disorders, viral diseases and postviral fatigue, body weight disorders, psychiatric disorders, cancer, cystic fibrosis, endometriosis, pre-menstrual syndrome, alcoholism, congenital liver disease, Alzheimer's syndrome, hypercholesterolemia, autoimmune disorders, atopic disorders, acute respiratory distress syndrome, articular cartilage degradation, diabetes and diabetic complications.
- 20 41. A method for diagnosing the presence of or a predisposition for a PUFA disorder in a subject by detecting a germline alteration in a sequence of claims 1 or 23 in said subject, comprising comparing the germline sequence of a sequence of claims 1 or 23 from a tissue sample from said subject with the germline sequence of a wild-type of said sequence, wherein an alteration in the germline sequence of said subject indicates the presence of or a predisposition to said PUFA disorder.
- 30 42. A method for diagnosing the presence of or a predisposition for a disorder as claimed in claim 41, wherein said disorder is selected from the group consisting of eczema, cardiovascular, inflammation, Sjögren's syndrome, gastrointestinal disorders, viral diseases and postviral fatigue, body weight disorders, psychiatric disorders, cancer, cystic fibrosis,
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endometriosis, pre-menstrual syndrome, alcoholism, congenital liver disease, Alzheimer's syndrome, hypercholesterolemia, autoimmune disorders, atopic disorders, acute respiratory distress syndrome, articular cartilage degradation, diabetes and diabetic complications.

5 43. The method of claims 41 to 42, wherein said comparing is performed by a method selected from the group consisting of immunoblotting, immunocytochemistry, enzyme-linked immunosorbent assay, DNA fingerprinting, in situ hybridization, polymerase chain reaction, reverse transcription polymerase chain reaction, radioimmunoassay, immunoradiometric assay and immunoenzymatic assay.

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44. A method for diagnosing the presence of or a predisposition for a PUFA disorder in a subject, comprising comparing the sequence of a polypeptide of claim 14 from a tissue sample from said subject with the sequence of a wild-type of said polypeptide, wherein an alteration in the sequence of said subject as compared to said wild-type indicates the presence of or a  
15 predisposition to said PUFA disorder.

45. A method for diagnosing the presence of or a predisposition for a disorder as claimed in claim 44, wherein said disorder is selected from the group consisting of eczema, cardiovascular, inflammation, Sjögren's syndrome, gastrointestinal disorders, viral diseases  
20 and postviral fatigue, body weight disorders, psychiatric disorders, cancer, cystic fibrosis, endometriosis, pre-menstrual syndrome, alcoholism, congenital liver disease, Alzheimer's syndrome, hypercholesterolemia, autoimmune disorders, atopic disorders, acute respiratory distress syndrome, articular cartilage degradation, diabetes and diabetic complications.

25 46. The method of claims 44 to 45, wherein said comparing is performed by a method selected from the group consisting of blotting, immunocytochemistry, enzyme-linked immunosorbent assay, DNA fingerprinting, radioimmunoassay, immunoradiometric assay, immunoenzymatic assay and polypeptide microarrays.

30 47. A method for identifying a compound which modulates a PUFA disorder, comprising identifying a compound which modulates the activity of a polynucleotide, wherein the polynucleotide is a coding sequence selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7, comprising the steps of:

(a) selecting a control animal having said polynucleotide and a test animal having said  
35 polynucleotide;  
(b) treating said test animal using a compound; and,

(c) determining the relative quantity of an expression product of said polynucleotide, as between said control animal and said test animal.

48. A method of claim 47, wherein said animals are mammals.

49. A method of claim 48, wherein said mammals are rats.

50. A method for identifying a compound which modulates a PUFA disorder, comprising identifying a compound which modulates the activity of a polynucleotide, wherein the polynucleotide is a coding sequence selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7, comprising the steps of:

(a) selecting a host cell having said polynucleotide, wherein said host cell is heterologous to said polynucleotide;

(b) cloning said host cell and separating said clones into a test group and a control group;

(c) treating said test group using a compound; and

(d) determining the relative quantity of an expression product of said polynucleotide, as between said test group and said control group.

51. A method for identifying a compound which modulates a PUFA disorder, comprising identifying a compound which modulates the activity of a polynucleotide, wherein the polynucleotide is a coding sequence selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7, comprising the steps of:

(a) selecting a test group having a host cell with said polynucleotide or a portion of said host cell, and selecting a suitable control group;

(b) treating said test group using a compound; and

(c) determining the relative quantity or relative activity of a product of said polynucleotide or of the said polynucleotide, as between said test group and said control group.

52. A method for identifying a compound modulates a PUFA disorder, comprising identifying a compound which modulates the activity of a polypeptide selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7, comprising the steps of:

(a) selecting a control animal having said polypeptide and a test animal having said polypeptide;

(b) treating said test animal using a compound;

(c) determining the relative quantity or relative activity of an expression product of said polypeptide or of the said polypeptide, as between said control animal and said test animal.

53. A method of claim 52, wherein said animals are mammals.

54. A method of claim 53, wherein said mammals are rats.

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55. A method for identifying a compound which modulates a PUFA disorder, comprising identifying a compound which modulates the activity of a polypeptide selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7, comprising the steps of:

(a) selecting a host cell comprising said polypeptide, wherein said host cell is heterologous to said polypeptide;

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(b) cloning said host cell and separating said clones into a test group and a control group;

(c) treating said test group using a compound; and

(d) determining the relative quantity or relative activity of an expression product of said polypeptide or of the said polypeptide, as between said test group and said control group.

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56. A method for identifying a compound which modulates a PUFA disorder, comprising identifying a compound which modulates the activity of a polypeptide selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7, comprising the steps of:

(a) selecting a test group having a host cell with said polynucleotide or a portion of said host cell, and selecting a suitable control group;

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(b) treating said test group using a compound; and

(c) determining the relative quantity or relative activity of a product of said polypeptide or of the said polypeptide, as between said test group and said control group.

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57. A method for identifying a compound which modulates the activity of a polynucleotide, wherein the polynucleotide is a control region of a gene selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7, comprising the steps of:

(a) selecting a control animal having said polynucleotide and a test animal having said polynucleotide;

30

(b) treating said test animal using a compound; and,

(c) determining the relative quantity of an expression product of an operably linked polynucleotide to said polynucleotide, as between said control animal and said test animal.

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58. A method of claim 57, wherein said animals are mammals.

59. A method of claim 58, wherein said mammals are rats.

60. A method for identifying a compound which modulates the activity of a polynucleotide, wherein the polynucleotide is a control region of a gene selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7, comprising the steps of:
- 5 (a) selecting a host cell comprising said polynucleotide, wherein said host cell is heterologous to said polynucleotide;
- (b) cloning said host cell and separating said clones into a test group and a control group;
- (c) treating said test group using a compound; and
- 10 (d) determining the relative quantity of an expression product of an operably linked polynucleotide to said polynucleotide, as between said test group and said control group.

61. A method for identifying a compound which modulates the activity of a polynucleotide, wherein the polynucleotide is a control region of a gene selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7, comprising the steps of:
- 15 (a) selecting a test group having a host cell with said polynucleotide or a portion of said host cell, and selecting a suitable control group;
- (b) treating said test group using a compound; and
- 20 (c) determining the relative quantity of an expression product of an operably linked polynucleotide to said polynucleotide, as between said test group and said control group.

62. A composition for treating a PUFA disorder comprising a compound which modulates a polynucleotide from the coding sequence selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6, and ELG7, and a pharmaceutically acceptable carrier.
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63. A composition for treating a PUFA disorder comprising a compound which modulates a polypeptide selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6, and ELG7, and a pharmaceutically acceptable carrier.
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64. A composition for treating a PUFA disorder comprising a compound which modulates a polynucleotide from the control region selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6, and ELG7, and a pharmaceutically acceptable carrier.

- 35 65. A composition as claimed in any one of claims 62 to 64, wherein said disorder is selected from the group consisting of eczema, cardiovascular, inflammation, Sjögren's syndrome, gastrointestinal disorders, viral diseases and postviral fatigue, body weight

disorders, psychiatric disorders, cancer, cystic fibrosis, endometriosis, pre-menstrual syndrome, alcoholism, congenital liver disease, Alzheimer's syndrome, hypercholesterolemia, autoimmune disorders, atopic disorders, acute respiratory distress syndrome, articular cartilage degradation, diabetes and diabetic complications.

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66. A composition as claimed in any one of claims 62 to 64, wherein said compound is selected from the group consisting of small organic molecules, peptides, polypeptides, antisense molecules, oligonucleotides, polynucleotides, fatty acids and derivatives thereof.

10 67. The use of a composition as claimed in any one of claims 62 to 64 for treating PUFA disorders.

15 68. The use of claim 67, wherein said disorder is selected from the group consisting of eczema, cardiovascular, inflammation, Sjögren's syndrome, gastrointestinal disorders, viral diseases and postviral fatigue, body weight disorders, psychiatric disorders, cancer, cystic fibrosis, endometriosis, pre-menstrual syndrome, alcoholism, congenital liver disease, Alzheimer's syndrome, hypercholesterolemia, autoimmune disorders, atopic disorders, acute respiratory distress syndrome, articular cartilage degradation, diabetes and diabetic complications.

20

69. A method for diagnosing the presence of or a predisposition for a PUFA disorder in a subject by detecting a germline alteration in a polynucleotide representing the coding sequence selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6, and ELG7, from said subject, comprising comparing the germline sequence of said polynucleotide from a  
25 tissue sample from said subject with the germline sequence of a wild-type of said polynucleotide, wherein an alteration in the germline sequence of said subject indicates the presence of or a predisposition to said PUFA disorder.

30 70. A method for diagnosing the presence of or a predisposition for a PUFA disorder in a subject by detecting a germline alteration in a polynucleotide representing the control region selected from the group consisting of ELG1, ELG2, ELG3 and ELG5 in said subject, comprising comparing the germline sequence of said polynucleotide from a tissue sample from said subject with the germline sequence of a wild-type of said polynucleotide, wherein an alteration in the germline sequence of said subject indicates the presence of or a predisposition  
35 to said PUFA disorder.



71. A method for diagnosing the presence of or a predisposition for a disorder as claimed in any one of claims 69 to 70, wherein said disorder is selected from the group consisting of eczema, cardiovascular, inflammation, Sjögren's syndrome, gastrointestinal disorders, viral diseases and postviral fatigue, body weight disorders, psychiatric disorders, cancer, cystic  
5 fibrosis, endometriosis, pre-menstrual syndrome, alcoholism, congenital liver disease, Alzheimer's syndrome, hypercholesterolemia, autoimmune disorders, atopic disorders, acute respiratory distress syndrome, articular cartilage degradation, diabetes and diabetic complications.
- 10 72. The method of claims 69 to 71, wherein said comparing is performed by a method selected from the group consisting of immunoblotting, immunocytochemistry, enzyme-linked immunosorbent assay, DNA fingerprinting, in situ hybridization, polymerase chain reaction, reverse transcription polymerase chain reaction, radioimmunoassay, immunoradiometric assay and immunoenzymatic assay.
- 15 73. A method for diagnosing the presence of or a predisposition for a PUFA disorder in a subject, comprising comparing the sequence of a polypeptide selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6, and ELG7, from said subject with the sequence of a wild-type of said polypeptide, wherein an alteration in the sequence of said  
20 subject as compared to said wild-type indicates the presence of or a predisposition to said PUFA disorder.
74. A method for diagnosing the presence of or a predisposition for a disorder as claimed in claim 73, wherein said disorder is selected from the group consisting of eczema,  
25 cardiovascular, inflammation, Sjögren's syndrome, gastrointestinal disorders, viral diseases and postviral fatigue, body weight disorders, psychiatric disorders, cancer, cystic fibrosis, endometriosis, pre-menstrual syndrome, alcoholism, congenital liver disease, Alzheimer's syndrome, hypercholesterolemia, autoimmune disorders, atopic disorders, acute respiratory distress syndrome, articular cartilage degradation, diabetes and diabetic complications.
- 30 75. The method of claims 73 to 74, wherein said comparing is performed by a method selected from the group consisting of immunoblotting, immunocytochemistry, enzyme-linked immunosorbent assay, DNA fingerprinting, radioimmunoassay, immunoradiometric assay, immunoenzymatic assay and polypeptide microarrays.
- 35 76. A method for identifying a compound which inhibits or promotes the overall activity of two or more polynucleotides, wherein the polynucleotides are control regions of two or

more different genes selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7, comprising the steps of:

(a) selecting a host cell having said polynucleotides, wherein said host cell is heterologous to said polynucleotides;

5 (b) cloning said host cell and separating said clones into a test group and a control group;

(c) treating said test group using a compound; and

(d) determining the relative quantities of expression products of operably linked polynucleotides to said polynucleotides, as between said test group and said control group.

10 77. A method for identifying a compound which inhibits or promotes the overall activity of two or more polynucleotides, wherein the polynucleotides are from control regions of said polynucleotides, selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7, comprising the steps of:

(a) selecting a test group having a host cell with said polynucleotide or a portion of said host

15 cell, and selecting a suitable control group;

(b) treating said test group using a compound; and

(c) determining the relative quantities of expression products of operably linked polynucleotides to said polynucleotides, as between said test group and said control group.

20 78. A method for identifying a compound which inhibits or promotes the activity of two or more polynucleotides, wherein the polynucleotides are coding sequences selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7, operably associated with promoter regions, wherein the promoter regions are effective to initiate, terminate or regulate the level of expression of the nucleic acid sequence, comprising the steps of:

25 (a) selecting a host cell having said polynucleotides, wherein said host cell are heterologous to said polynucleotides;

(b) cloning said host cell and separating said clones into a test group and a control group;

(c) treating said test group using a compound; and

30 (d) determining the relative quantity or relative activity of an expression product of said polynucleotide, as between said test group and said control group.

79. A method for identifying a compound which inhibits or promotes the activity of two or more polynucleotides, wherein the polynucleotides are coding sequences selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7, operably associated

35 with promoter regions, wherein the promoter regions are effective to initiate, terminate or regulate the level of expression of the nucleic acid sequence, comprising the steps of:

- (a) selecting a test group having a host cell with said polynucleotide or a portion of said host cell, and selecting a suitable control group;
- (b) treating said test group using a compound; and
- (c) determining the relative quantity or relative activity of an expression product of said polynucleotide, as between said test group and said control group.

80. A method for identifying a compound which inhibits or promotes the activity of a mammalian delta-5-desaturase enzyme and one or more enzymes selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 or ELG7, within the same host system, comprising the steps of:
- (a) providing a host system containing nucleic acid sequences which encode for a mammalian delta-5-desaturase and one or more mammalian elongase enzymes selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 or ELG7, operably associated with promoter regions, wherein the promoter regions are effective to initiate, terminate or regulate the level of expression of the nucleic acid sequence;
  - (b) contacting the host system with a test component;
  - (c) simultaneously evaluating the enzymatic activities of the delta-5-desaturase and the elongase enzymes, wherein a measurable difference in a level of lipid metabolites or associated cofactors in the presence of the test component compared to a control under identical conditions but in the absence of the test component is an indicator of the ability of the test component to modulate delta-5-desaturase and/or elongase enzyme activity; and
  - (d) identifying as said compound a test component which exhibits said ability.

81. A method for identifying a compound which inhibits or promotes the activity of a mammalian delta-6-desaturase enzyme and one or more enzymes selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 or ELG7, within the same host system, comprising the steps of:
- (a) providing a host system containing nucleic acid sequences which encode for a mammalian delta-6-desaturase and one or more mammalian elongase enzymes selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 or ELG7, operably associated with promoter regions, wherein the promoter regions are effective to initiate, terminate or regulate the level of expression of the nucleic acid sequence;
  - (b) contacting the host system with a test component;
  - (c) simultaneously evaluating the enzymatic activities of the delta-6-desaturase and the elongase enzymes, wherein a measurable difference in a level of lipid metabolites or associated cofactors in the presence of the test component compared to a control under

identical conditions but in the absence of the test component is an indicator of the ability of the test component to modulate delta-6-desaturase and/or elongase enzyme activity; and  
(d) identifying as said compound a test component which exhibits said ability.

5 82. A method for identifying a compound which inhibits or promotes the activity of a mammalian delta-5- and delta-6-desaturase enzyme and/or one or more enzymes selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 or ELG7, within the same host system, comprising the steps of:

(a) providing a host system containing nucleic acid sequences which encode simultaneously  
10 for a mammalian delta-5-desaturase, a mammalian delta-6-desaturase and one or more mammalian elongase enzymes selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 or ELG7, operably associated with promoter regions, wherein the promoter regions are effective to initiate, terminate or regulate the level of expression of the nucleic acid sequence;

15 (b) contacting the host system with a test component;

(c) simultaneously evaluating the enzymatic activities of the delta-5-desaturase, the delta-6-desaturase and the elongase enzymes, wherein a measurable difference in a level of lipid metabolites or associated cofactors in the presence of the test component compared to a control under identical conditions but in the absence of the test component is an indicator of  
20 the ability of the test component to modulate delta-5- and/or delta-6-desaturase and/or elongase enzyme activity; and

(d) identifying as said compound a test component which exhibits said ability.

83. A composition for treating a PUFA disorder comprising a compound which modulates  
25 two or more human polynucleotides from control regions selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6, ELG7, delta-5-desaturase, delta-6-desaturase and a pharmaceutically acceptable carrier.

84. A composition as claimed in claim 83, wherein said compound is selected from the  
30 group consisting of small organic molecules, peptides, polypeptides, antisense molecules, oligonucleotides, polynucleotides, fatty acids and derivatives thereof.

85. The use of a composition as claimed in claim 84 for treating PUFA disorders.

35 86. The use of claim 85, wherein said disorder is selected from the group consisting of eczema, cardiovascular, inflammation, Sjögren's syndrome, gastrointestinal disorders, viral diseases and postviral fatigue, body weight disorders, psychiatric disorders, cancer, cystic

fibrosis, endometriosis, pre-menstrual syndrome, alcoholism, congenital liver disease, Alzheimer's syndrome, hypercholesterolemia, autoimmune disorders, atopic disorders, acute respiratory distress syndrome, articular cartilage degradation, diabetes and diabetic complications.

5

87. A method for detecting the presence of or the predisposition for a PUFA disorder, said method comprising determining the level of expression of two or more expression products of genes selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6, ELG7, delta-5-desaturase, delta-6-desaturase, in a subject relative to a predetermined control level of expression, wherein any modified expression of said expression products as compared to said control is indicative of the presence of or the predisposition for a PUFA disorder.

10

88. A method of claim 87, wherein said disorder is selected from a group consisting of eczema, cardiovascular, inflammation, Sjögren's syndrome, gastrointestinal disorders, viral diseases and postviral fatigue, body weight disorders, psychiatric disorders, cancer, cystic fibrosis, endometriosis, pre-menstrual syndrome, alcoholism, congenital liver disease, Alzheimer's syndrome, hypercholesterolemia, autoimmune disorders, atopic disorders, acute respiratory distress syndrome, articular cartilage degradation, diabetes and diabetic complications.

15

20

89. A method of claims 87 to 88, wherein said method is selected from the group consisting of immunoblotting, immunocytochemistry, enzyme-linked immunosorbent assay, in situ hybridization, reverse transcription polymerase chain reaction, radioimmunoassay, immunoradiometric assay, immunoenzymatic assay and polynucleotide and polypeptide microarrays.

25

90. An antibody immunoreactive with a polypeptide of claim 14 or an immunogenic portion thereof.

30

91. An antibody immunoreactive with an elongase polypeptide selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7, or an immunogenic portion thereof.

35

92. A method for screening a medium for an elongase polypeptide of claim 14 or selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7, comprising;

- (a) labelling an antibody of any one of claims 90 to 91 with a marker molecule to form a conjugate;  
(b) exposing said conjugate to said medium; and  
(c) determining whether there is binding between said conjugate and a biomolecule in said medium, wherein said binding indicates the presence of said polypeptide.

93. A method for screening a medium for an elongase polypeptide of claim 14 or selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7, comprising:

- (a) exposing an antibody of claims 91 to 92 to said medium;  
(b) exposing said antibody to a marker molecule; and  
(c) determining whether there is binding between said marker molecule and a biomolecule in said medium, wherein said binding indicates the presence of said polypeptide.

94. A composition as claimed in claim 36, wherein said compound is selected from the group in claim 90.

95. A composition as claimed in any one of claims 62 to 64, wherein said compound is selected from the group consisting of antibodies against ELG1, ELG2, ELG3 and ELG5.

96. The use of a composition as claimed in any one of claims 94 to 95 for treating a PUFA disorder.

97. The use of claim 96, wherein said disorder is selected from the group consisting of eczema, cardiovascular, inflammation, Sjögren's syndrome, gastrointestinal disorders, viral diseases and postviral fatigue, body weight disorders, psychiatric disorders, cancer, cystic fibrosis, endometriosis, pre-menstrual syndrome, alcoholism, congenital liver disease, Alzheimer's syndrome, hypercholesterolemia, autoimmune disorders, atopic disorders, acute respiratory distress syndrome, articular cartilage degradation, diabetes and diabetic complications.

98. A compound identified by the methods of any one of claims 9 to 13, 18 to 22, 31 to 35, 47 to 61 or 76 to 82.

99. The use of a compound as claimed in claim 98 for treating a PUFA disorder.

100. The use as claimed in claim 99, wherein said disorder is selected from the group consisting of eczema, cardiovascular, inflammation, Sjögren's syndrome, gastrointestinal disorders, viral diseases and postviral fatigue, body weight disorders, psychiatric disorders, cancer, cystic fibrosis, endometriosis, pre-menstrual syndrome, alcoholism, congenital liver disease, Alzheimer's syndrome, hypercholesterolemia, autoimmune disorders, atopic disorders, acute respiratory distress syndrome, articular cartilage degradation, diabetes and diabetic complications.
101. A method for diagnosing the presence of or a predisposition for a PUFA disorder in a subject by detecting alterations as compared to wild-type in the elongation of PUFA in a peripheral blood leukocyte obtained from said subject.
102. A method for monitoring the development of a PUFA disorder in a subject by detecting alterations as compared to previous samples in the elongation of PUFA in a peripheral blood leukocyte obtained from said subjects.
103. A method for assessing the efficacy of test compounds on a PUFA disorder in a subject by assessing alterations as compared to previous samples in the elongation of PUFA in a peripheral blood leukocyte obtained from said subject.
104. The use of pebulate sulphoxide for the treatment of a disease selected from the group consisting of eczema, cardiovascular, inflammation, Sjögren's syndrome, gastrointestinal disorders, viral diseases and postviral fatigue, body weight disorders, psychiatric disorders, cancer, cystic fibrosis, endometriosis, pre-menstrual syndrome, alcoholism, congenital liver disease, Alzheimer's syndrome, hypercholesterolemia, autoimmune disorders, atopic disorders, acute respiratory distress syndrome, articular cartilage degradation, diabetes and diabetic complications.
105. A method for identifying a compound which modulates a biological activity of a polypeptide selected from the group consisting of ELG1, ELG2, ELG3, ELG4, ELG5, ELG6 and ELG7, comprising the steps of: (a) providing an assay which measures a biological activity of the selected polypeptide; (b) treating the assay with a compound; and (c) identifying a change in the biological activity of the selected polypeptide, wherein a difference between the treated assay and a control assay identifies the compound as modulator of the polypeptide.

106. The method of claim 105, wherein the selected polypeptide is provided in an assay format selected from among a purified protein, reconstituted protein, cell extract and a whole cell assay.
- 5 107. The composition as claimed in any one of claims 37 and 65, wherein the cardiovascular disorder is selected from the group consisting of hypertriglyceridemia, dyslipidemia, atherosclerosis, coronary artery disease, cerebrovascular disease and peripheral vascular disease.
- 10 108. The use as claimed in any one of claims 40, 68, 86, 97, 100, and 104, wherein the cardiovascular disorder is selected from the group consisting of hypertriglyceridemia, dyslipidemia, atherosclerosis, coronary artery disease, cerebrovascular disease and peripheral vascular disease.
- 15 109. The method as claimed in any one of claims 42, 45, 71, 74, and 88, wherein the cardiovascular disorder is selected from the group consisting of hypertriglyceridemia, dyslipidemia, atherosclerosis, coronary artery disease, cerebrovascular disease and peripheral vascular disease.
- 20 110. The composition as claimed in any one of claims 37 and 65, wherein the inflammation is selected from the group consisting of sinusitis, asthma, pancreatitis, osteoarthritis, rheumatoid arthritis and acne.
- 25 111. The use as claimed in any one of claims 40, 68, 86, 97, 100, and 104, wherein the inflammation is selected from the group consisting of sinusitis, asthma, pancreatitis, osteoarthritis, rheumatoid arthritis and acne.
- 30 112. The method as claimed in any one of claims 42, 45, 71, 74, and 88, wherein the inflammation is selected from the group consisting of sinusitis, asthma, pancreatitis, osteoarthritis, rheumatoid arthritis and acne.
113. The composition as claimed in any one of claims 37 and 65, wherein the body weight disorder is selected from the group consisting of obesity, cachexia and anorexia.
- 35 114. The use as claimed in any one of claims 40, 68, 86, 97, 100, and 104, wherein the body weight disorder is selected from the group consisting of obesity, cachexia and anorexia.



115. The method as claimed in any one of claims 42, 45, 71, 74, and 88, wherein the body weight disorder is selected from the group consisting of obesity, cachexia and anorexia.

FIGURE 1

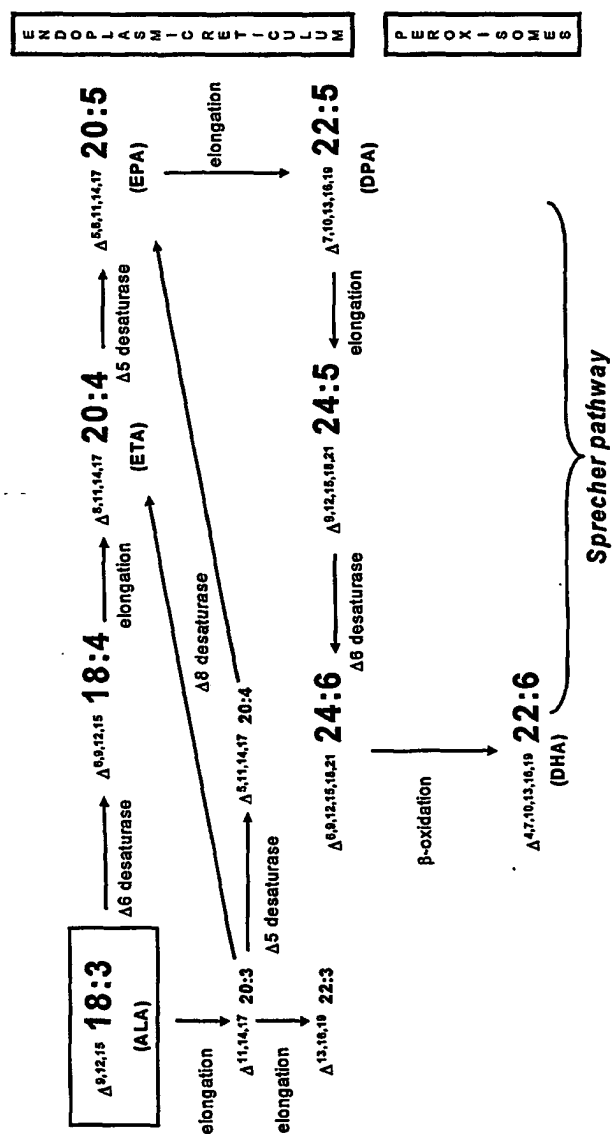


FIGURE 2

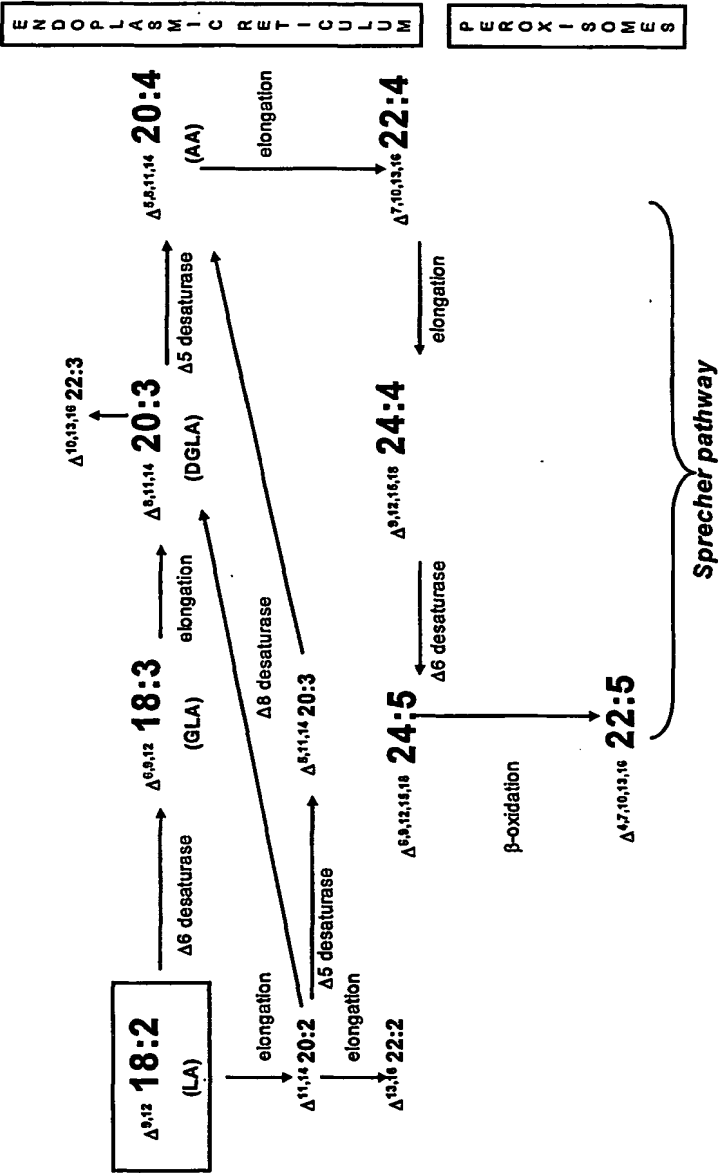


FIGURE 3

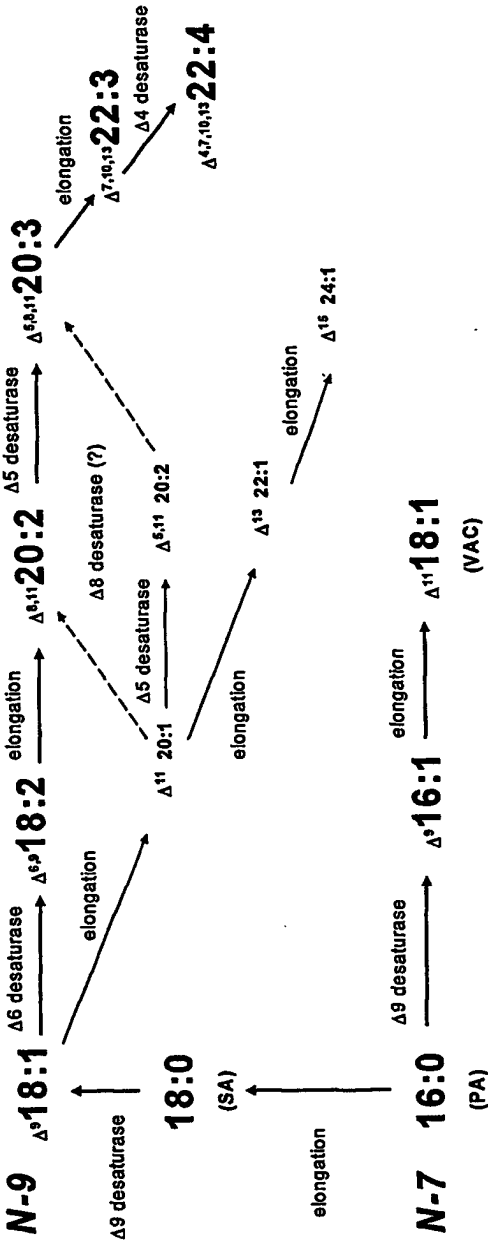


FIGURE 4

ELG1 -----MEAVNLYQEVKMHADPRIQGYPLMGSPLLMTSILLT 37  
 ELG2 -----MEHFDASLSTYFKALLGPRDTRVKGNFLDN-VIPTFICSV 40  
 ELG3 -----MEHLKAFDEINAFLDNMFGRDPSRVRGWFMELDS-YLPTFFLTV 43  
 ELG4 -----MAFSDLTSRTVHLTDNWKIDADPRVEDNLLMSSPLPQWILIGF 43  
 ELG5 -----MNMVLTLOEYEFKQFNENEAIQWQENWKKSEFESA 38  
 ELG6 -----MVTAMVSHENVQLFQPINFELSMDRPFEEYWAISEPIAL 42  
 ELG7 MGLDSEPGSVLNVVSTALNDTVEFTRWTWSIADKRVENWPLMQS-PWPTLSIST 54

\* \* \*

ELG1 YVYFVLSLGPRIIMANRKPQLRGFMIVYNESIVALSLY-TVYEFMLSGWLSYTW 91  
 ELG2 TYLLIVNLGPKYMRNKQPFSCRGILVYNLGLTLLSLY-MFCELVTGVWEGKYNF 94  
 ELG3 MYLLSIVNLGNKYMRNPALSIRGILTYNLGTYLLSAY-MYAEILISTWEGGYNL 97  
 ELG4 YVYFVTSLGPKLIMENRKPFEELKKAMITYNFIVLEFSVY-MCYEFFMMSGNGICYSF 97  
 ELG5 IYAAFIYFGRHIMNKRKAKFELRKPLVLSLTIAVFSIFGALRTGAYMYIIMTKG 93  
 ELG6 IYLVLLTAVGQNYMKEKGFNLQGPLILWSECLAFSILGAVRMWGMGTVLLTGG 97  
 ELG7 LYHLEFVNLGPKMKDREPTQMRVLVLIYNEFGMVLDLNF-DFRELFWGSKXNAGYSY 108

\*\* \* \*\* \*\* \*

\*\* \* \*\* \*\* \*

ELG1 RCDPVDYNSPEALRMVRVAMLELEFSKFIELMDTVIFILRKKGQVTFILHVFFHS 146  
 ELG2 FCQGTRTAG-ESDMKIIIRVLNWWYFESKLTIEFMDTFFFILRKNHQTIVLHVYHHA 148  
 ELG3 QCQDLTSAG-EADIRVAKVLMWYFESKSVLELDTIFFVLRKKTISOITFLHVYHHA 151  
 ELG4 RCDIVDYRSRPTALRMARTCMLYFESKFIELDTIFFVLRKKNQVTFILHVFFHT 152  
 ELG5 LKQSVCDQGFYNGPVSKFWAYAVL SKAPELGDITIFILRKQ--KLIFLHWYHHI 146  
 ELG6 LKQTVCFINFDNSTYKFWSNVVELLSKVLELGDITILRKR--PLIFLHWYHHS 150  
 ELG7 ICQSVDISNNVHEVFAAALMWYFVSKGVEYLDTVFFILRKNQVSTFLHVYHHC 163

FIGURE 4 (continued)

ELG1	VLPWSNWWGVKIALPGGMGSEFHAMINSVHVIMLYLYGLSAGFVAQPYLWKKHM	201
ELG2	SMNLNWEVNMVPCGHSYFCATLNSFTHVMYSYGLSSV-PSMRPFLWKKYL	202
ELG3	SMFNINWCVLNWPCCQSEFGPTINSETHILMYSYGLSVF-PSMHKYLWKKYL	205
ELG4	IMPWTWFGVKFAAGGLGTFHALINTAVHVMYSYGLSALCBAYQKYLWKKYL	207
ELG5	TVLLYSWYSYKDMVAGCGWE-MTMNYGVHAVMYSYALRAA--GFRVSRKFAMFL	198
ELG6	TVLVYTSFGYKKNVPAGGWE-VTMNFGVHAIMYTYVTLKRA--NVKPPKMLPMLL	202
ELG7	TMFTLWIGIKWVAGGQAFEGAQLNSFTHVIMYSYGLTAFGFWIQKYLWKKYL	218

ELG1	TALQLIQFVLVSLHISQYFMSSQNYQYPVLIHLIWM--YGTIFFMLESNEWYHS	254
ELG2	TQQLIQFVLTIIQTSQVWP-CIEPLGWLIFQIG--YMSISLALFTNEFYIQT	253
ELG3	TQQLVQFVLTITHTMSAVKP-CGEPFGCLIFQSS--YMLTLVILFLNFIYVQT	256
ELG4	TSLQLVQFVIVAIHISQFFEMEDCKYQFPVFACIIMS--YSFMELLLFLHFHYRA	260
ELG5	TLSQITQMLMGCVVNVLVFCWQHDOCHSHFQNDFWSSILMYSYLVLFCHFFFEA	253
ELG6	TSLQITQMFVGAIVSILTYIWRQDQGGCHTMEHDFWSEFLYMYFILLFAHFFCQT	257
ELG7	TMLQLIQFHVTIQHTALSLYTD-CPEPKWMHWALIA--YASIEFLFLNFIYIRL	269

ELG1	YTKG-KRLPRALQONGAPGLAKVRAN-----	279
ELG2	YNKKGASRRKDHDKDHONGSMAAVNGHTNSFSPLENNVKPKLRKD--	299
ELG3	YRKK--PMKKDMQEP--AGKEVKNQFSKAYFTAANGVMNKKRAQ----	296
ELG4	YTKG-QRLPKTVKN---GTCKNRDN-----	281
ELG5	YIG---KMRKTTKAE-----	265
ELG6	YIRP--KVKAKTKSQ-----	270
ELG7	YKEP---KKPKAGKTAMNGISANGVSKSEKQLMIENTKKQKNGKAKGCD	314

FIGURE 5

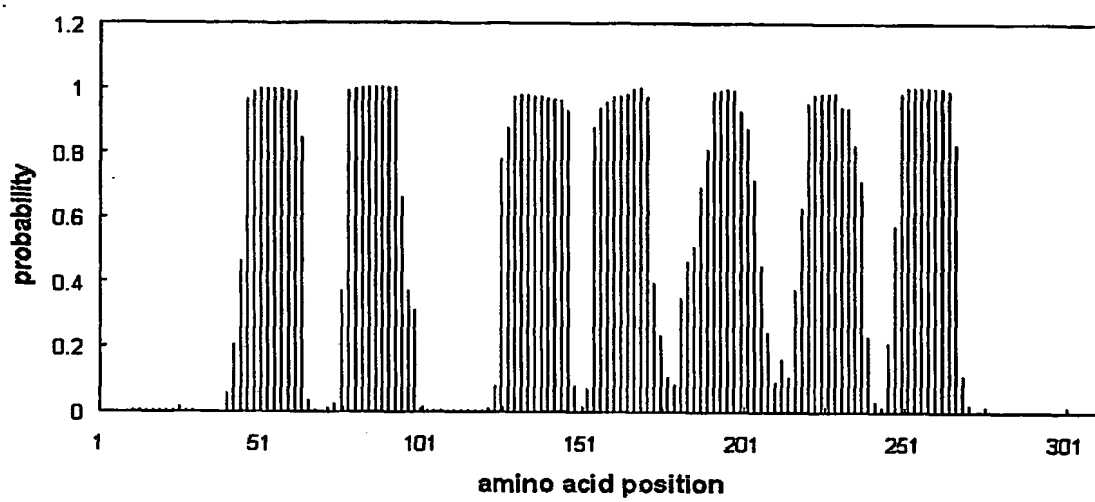


FIGURE 6

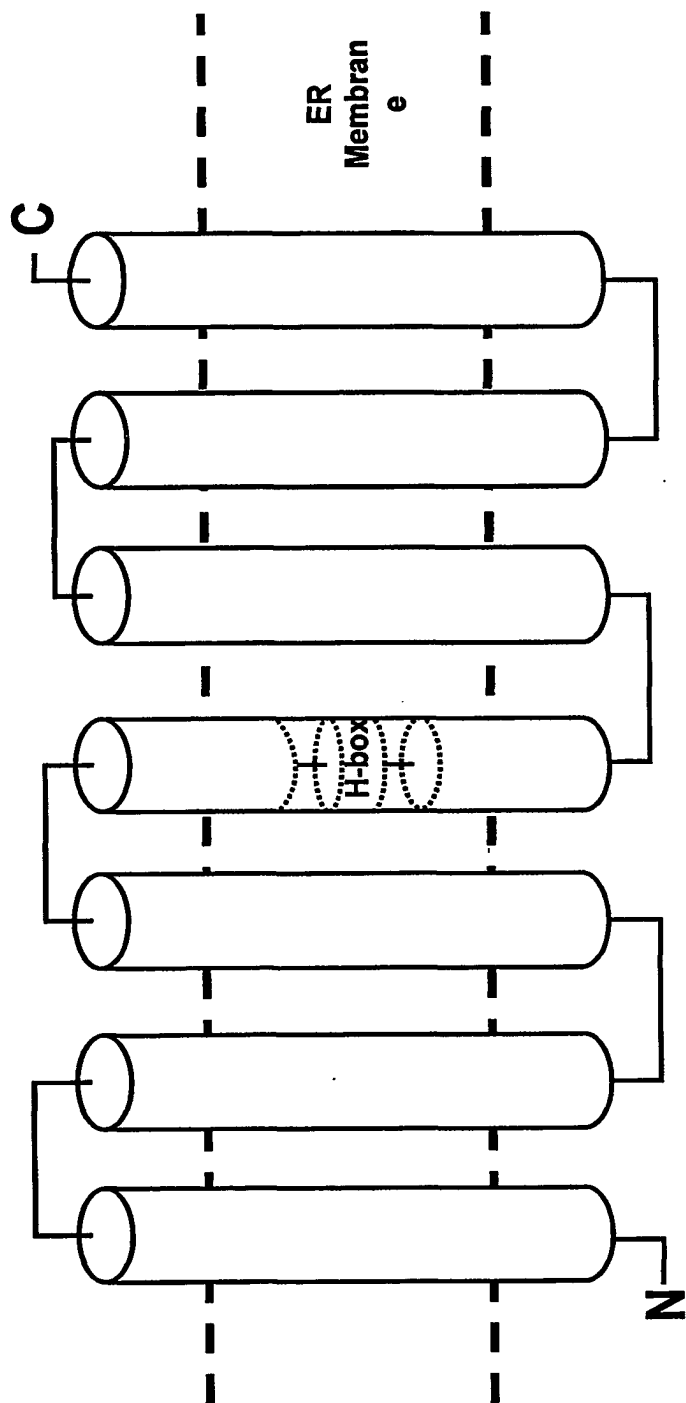




FIGURE 7

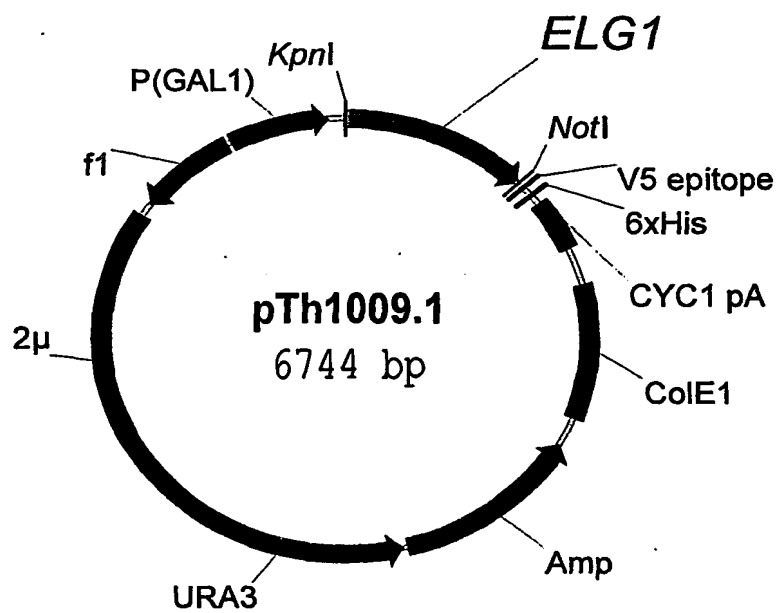


FIGURE 8

1 TACAGGCTCG TGAGGCTTCC CTCCCGCTAA GACCAGTGCG CCCTCAGCAC  
51 ACGCAGTGTG GTCTCGCCCG CCGCTCTGCG CTCGCCCTGC AGGAGAGGGA  
101 GCTCTTTGAA GGCAAGGCCG AACCTCCCCC GAGCCCTGAG CTGGGCCTGC  
151 CGCCACAGAT GTGCAGTCCT GCCGGGGAGC AGTCACCCGG GGACAGGGCC  
201 GGGCCCCGGG CTGCACGTCG GGAAGAGACA GCGTGCTCCT GAGGTGGCCA  
251 GGCCGCTGCA ACTGGCCAGG GCGGGCCCGG GCGGCGAGGG AAGGGGTGGG  
301 AAGCCCGGGC CGCGGCGCTT CCTGCTGGGA CCCGGCGGCA CGCCCCTGCC  
351 CCCGCCCCGG CCGAGCCTGC GCTGCCGGCC TCCGGCCCTG CCGGCCGCCC  
401 AATCAGCGGG CGCCCCCGC GCGGCCCCGCC CCTCCCCCTC TGGTGACAGA  
451 AAGTCGGCCC AGCAGATGAG GAAGTGGCAG GCAGGCAGGC TGGCCCCGGG  
501 GACTTCTCTC TGGCCCTGCT CCCTCCGAGC GCTCCGCCGT TGCCCGCCTG  
551 GCCCCTACGG GTGAGTCTGG ACCTTCCACG GACTCTCCAC GTGCCGGCGC  
601 CCCCTGCCTG GCCAGCCCGG CCCAGCCCGG CCCAGCCCTG CCCTGCCCTG  
651 CCCAGGCTGT GGGCGAGGGT GTTCCCGGGG CCAGTGGGTG GGAGGTCCCA  
701 GCTCCCTGGG GCCGGGCCTC GCCAGCACCC TCCCTCCCCC ACACCCCCGT  
751 CTCTGGCCCC CATTTGCCTA CACCCGGGCC TTCCTCCACC ACCCCTGCAT  
801 TTACCTCTCT CCCTCCTCCT CTCCCCTCCC TCCCCCGCT ACCCTAACTT  
851 TGCCAGGCAC CTTTTCCTT CCATCCATCT TAAAGGAAGG AAGGGACGGG  
901 CTGAGTCCC CGACGAGAGA CACACCCAGA TTTTCCTGCA GCTTGGGGAG  
951 AGGTCCTCCC AGGAGCCTTG GTCCCTCCTG GCCTGCCGG

FIGURE 9

1 CGAGGGTGGG CTTCTGCCAC CCAAATGCGG CCACAGACTC CTGCCACGCC  
51 TGGCAGTAAA AAAACCAGAG TTCAGGGCAT CGACAACTTC ACCGGGGCTA  
101 TTGCGCAGGC TCTGCGTTCC ACGCAGGCTT ATTAGGAAGA AAGGGGAAAA  
151 AAATTTCCCA GAGACACGTG GAACCGAGGG GCCAACCCCG GCCTAGGCTC  
201 TCCACCGCAT CGGATTCTGG AATTACGAT CACGAAAGTT CTATTGTCCC  
251 GCGATTGGCT CCCGGGCCGC ATGACATCAT AGCGCTTGAT TCATCCTTCG  
301 GGTCCCGATT GGCTGGCCGC GCCATTGTGA CGTCACGGTC AGCCCACGTT  
351 CTGATTGTAG ATAGCCGGCG CCTTCCTCTT CCCATCGCGC GGGTCCTAGC  
401 CACCGGTGTC TCCTTCTACA TCCGCCTCTG CGCCGGCTGC CACCCGCGCT  
451 CCCTCCGCCG CCGCCGCCTT GCTGCTGCTC AAAGCTGCTG CCGCCCCTTG  
501 GGCTAAAAG

FIGURE 10

1 CCGGTACCTA CAGTTACTCA CTCTGCTACT GCACAAAACT CTGCAAGGGC  
51 TCCCACACCG CCCAGGTGT GGGATGCTAA GTGTATGGTG CAGGTACCTC  
101 CGTGACACAG CACACGGGCT GCTCTCAACC CCAATAAACA TGTTTACCAC  
151 ATGAGCCTCA CATGTGGTAA ACATTTTTTT TTTTTTTTTT TTTTTTTTGA  
201 GACAGGGTCT CATTCTGTCG CCCAGGCTGG AGTGCAGTGG CGTGATCTCG  
251 GCTCACTGCA GCCTCCACCT CCAGGGCTCA AGCCATCCTT CACCTCAGCC  
301 TCCCGAGTGG CTGGGATCAC AGGCGCAGGC CACCACACCC AGCTAATTTT  
351 TGTATTTTTT ATTTAAGAGG CGGGGTTTCG CCATGTTGCC CAGGCTGGTC  
401 CCGAACTCCT GACCTCAAGT GATTCGCCTG CCTCAGCCTC CCAAAGTGCT  
451 GGGATTACAG GAGGGAACCA CCACGCCCCG CAACTTCCCA TGCTTGAGGG  
501 AGAAATGGAA GAAAGTTCAT GTAATACTCA GGCAAGTCCA ATTTTTTCGA  
551 CGTCTTTCAC TTGGGCCACA CACACAATA AAGTAACTAG AAGCGCAGGC  
601 TCTAGGAGGC CACCGTTCTG TTCACAGTGA AGAGGGTGCG CTCACCGTTG  
651 GTCGTGTCCG CTGGAAGCCC CGCGTCAGGC CGGGAGCGGG ACAGAGACTC  
701 TTGCTCAGGG CCGTTATCCG AACTGATCCG CTTCCCACCG CACCCCCAGA  
751 GAAACCCACC CAACCCCTA AACCTAAGAA ACCCAGACTG CGCAAACCTG  
801 CAGGAACAGA GCCATTTCCC CCTAATGTGT GCTTCAAACC CACCGAAGCC  
851 CAACTGTAAG CAAGACCAGC GTGCCCCGCC TGCACGATAC TGCTTCTCCC  
901 CGCAGCAGCG GCTGCCGATC TGGGCAGCGG GTGGGTATTC CTGGGGCTCC  
951 GTGGACGTTG AGCCGCCGCG CGAAACCGGC GCCGGCTGGA CCTGCAAATC  
1001 GCCGCCCGGC CGGCAGGGGA CGCCGCGGAC GCGAGGGCGA GGTCCGGTCG

FIGURE 10 (continued)

1051 CCAGGAGGGG GCGCGCGAGG CCGCAGGGGC GGGGGGCGCC GCCTCACTTG  
1101 CCCTGCGCCC CTCCCCGCG CGCCCTCCTG GCGCGGCGGC CGGCGAGGCC  
1151 CCTGTGGGAG AGGGGGCGGG GACGAAACGG CCCCAGGGCT CGGAGCGCCG  
1201 CGCGGCGGCG GCGCGAGCCC GAGGGGGCGG GGAGGCGCGG GCGGGTGTGC  
1251 GCGCGCCGGG CGTGGGTGTG GGTGGGGGTA ACCGGCGCGG GCGCCGAGAT  
1301 AGCGCCGGGC AGAGGGACCC GGCTACCCTG GACAGCGCAT CGCC

FIGURE 11

5' ATG GCC TTC AGT GAT CTT ACA TCG AGG ACT GTG CAT CTT TAT GAT AAT TGG ATC  
 M A F S D L T S R T V H L Y D N W I

60 AAA GAT GCT GAT CCA AGA GTT GAA GAT TGG CTC CTC ATG TCC TCG CCT CTG CCA  
 K D A D P R V E D W L L M S S P L P

120 CAA ACC ATC CTC CTA GGA TTC TAT GTC TAT TTT GTC ACT TCC TTG GGA CCA AAG  
 Q T I L L G F Y V Y F V T S L G P K

180 CTC ATG GAA AAT CGC AAG CCC TTT GAA CTC AAG AAA GCA ATG ATA ACG TAC AAT  
 L M E N R K P F E L K K A M I T Y N

240 TTT TTC ATA GTA CTC TTT TCT GTG TAT ATG TGT TAT GAG TTT GTG ATG TCT GGC  
 F F I V L F S V Y M C Y E F V M S G

300 TGG GGT ATA GGT TAT TCA TTT CGA TGT GAC ATT GTT GAC TAT TCA CGG TCA CCC  
 W G I G Y S F R C D I V D Y S R S P

330 ACA GCT TTG AGG ATG GCA CGT ACC TGC TGG CTT TAT TAC TTC TCC AAA TTT ATT  
 T A L R M A R T C W L Y Y F S K F I

390 GAG CTA TTA GAT ACG ATC TTT TTT GTT CTG CGC AAG AAA AAT AGC CAA GTG ACT  
 E L L D T I F F V L R K K N S Q V T

450 TTC CTT CAT GTA TTC CAT CAT ACC ATC ATG CCG TGG ACC TGG TGG TTT GGA GTC  
 F L H V F H H T I M P W T W W F G V

510 AAA TTT GCT GCA GGT GGT TTG GGA ACA TTC CAT GCC CTT CTA AAT ACA GCT GTA  
 K F A A G G L G T F H A L L N T A V

570 CAT GTA GTC ATG TAT TCC TAC TAT GGA CTT TCT GCA TTG GGG CCA GCC TAC CAG  
 H V V M Y S Y Y G L S A L G P A Y Q

FIGURE 11 (continued)

600  
 AAG TAT TTG TGG TGG AAA AAA TAT TTG ACA TCA TTA CAG CTT GTC CAG TTT GTT  
 K Y L W W K K Y L T S L Q L V Q F V

660  
 ATT GTC GCC ATC CAC ATA AGC CAG TTC TTT TTC ATG GAG GAT TGC AAG TAT CAG  
 I V A I H I S Q F F F M E D C K Y Q

720  
 TTT CCA GTC TTT GCG TGC ATC ATT ATG AGT TAC AGT TTC ATG TTT CTG CTG CTC  
 F P V F A C I I M S Y S F M F L L L

780  
 TTT CTC CAT TTT TGG TAC CGT GCT TAC ACC AAA GGT CAG AGG TTG CCC AAA ACT  
 F L H F W Y R A Y T K G Q R L P K T

840  
 GTG AAA AAT GGA ACT TGC AAA AAC AAA GAT AAT TGA 3'  
 V K N G T C K N K D N \*

FIGURE 12

1 TCGGCCTGGC TGAACACTAC ATTTTTTTTA CTTCTTTATT CATGTATTGT  
51 CTGTCATCTC CAACTAGAAT GAACGTATAG TCCCTGAGAA CGGGGAATTT  
101 GTTATCTATT GAAACTTCAG GGCCTGGAAC ATAGCAGCAC TCCAGTATTT  
151 GTTAAATAAA TGAATCCATT TGAGCTTCTG CATATTTGAA ATTCATAAG  
201 TATATATAAA TGGTAAATTG TGATAGACTC AAAGGCTAGT ATCATTAGGC  
251 AATTGTCTCC CGTTCCCAA AGACTTCCTA AGTCTACTAA ATGATCTGTT  
301 TTTAATATGA AAGCAAAGTT ATCTAAAAGA AAGGAGAAAT CTTTAGTTTT  
351 TTTGACTTCG AGATTCTTTG CAATTTAAGC TTTTTTTTTT TTTTTTTTTT  
401 TTTTTTTTTG CTTTCTTTC AATGGACACT TTCGAAGTTT TACATAAAAA  
451 CATTAAACC TCTTGTTTAA TGTAGTGGGA TTAAGCTGCC GAAGGCAATC  
501 CCTACATGTG AGGAAAATAT GCTTCCGACA CCCCAATTTT TTTTTCTCC  
551 CTACCCATCC TCTCTGGTGG TCCTGACGCT CCCAGCCCCT TTTTGTGTTT  
601 CTTGATTCCA TGCTGAGAAC TCGCAATACA AACTCAAAGC CCACATTTGT  
651 GAGGTGGTTG GGTCAGGACT GCAACTAAAA ATGATTATTG TTTTTTAGGT  
701 TTCTGGACAG TTCAACACCA GCCTTTGGTT TTGCCTCAGA AGCAGGGAAC  
751 TTCTCTAGGC CCCTATTTTG CCTTTCAGCT ATTGATGATC CAAATCATAC  
801 CAGCGATTAG GAGGATCATT ACCAGACACA AGGCCAGGTA CGTTTAAAAA  
851 ATAAATAAAC CAAGCGCAGG TGCACACTCC GAACGCTCAT CCCCACCCCC  
901 ACTTTCCAAT CCAACAGTAG GTAACGAGAA ATGAATTTTC TAGACTTTTT  
951 TTCCTGCAGC AGTTGCTGTT ACCAGAAACA AAGTTAGATG ATATACAATC  
1001 TAATCTTCAT TGCTCTAAAA GTCTCTCCC CATGCCCCC AGGCTGCCTC



FIGURE 12 (continued)

1051 AATTCTCTAG TTTCTTATTC CTTATAAGCA GGGGATGGAG CTGAACCAAG  
1101 TCGGCCTTCC CCTCCCAGGG CTTTCTCCTC TTGGTCTGGC TTCCATTTC A  
1151 GATGCGAATT AACCCCTCCA ATACCCTTTC AGAAGCAAGG AGTCCCCTTT  
1201 TTCTCCGCCT CCAGCCTCAG CTAGGTTTTT CTCATTTTCG ATTTTTCTAC  
1251 AGCTCATTC CAAATGAGTC ACGCATGACG ACAATTTCCA CTCTGCTATG  
1301 TCAGCCTGGA GATGTCCCCC AAGTGATGGC ATCTGCTCTC GGAAAGAAAG  
1351 GTCATCGGTG CCACGACCAG CCCCCTAAC CCAGAGCGGC CGGTGGGCCC  
1401 CAGTCCCGAG AGTCAGGGCG CGCGGCGGAG GCGAGGCCGG GCGGCGCTCC  
1451 GCCCTCCCGG CCGCTCCCCC TCGCGCCGCC CCGGCTCCTC CCTCCGGCCC  
1501 TCGGCGGGCA CCTGGCGGCG GCGGGCAGGG GCGGCGCTG CGCGCGTCAC  
1551 GCGGCTGGGT GGGATAGCGG GCAGGTGACA CCCGGCGGCC TCCTCCCCTT  
1601 TCCAACCCAG TCGGCGGCCG GGACAGCAGG GGCCGCTGTG AGGAGCTCCG  
1651 CGCTCGCGCT GCCAGTCGCC GCCCTCTCTC CCGCGCGCGC CCGGCGCTTC  
1701 GGCTCCGCTC CCTGTGCGGT GAGTGCGGGG TTCCAGGCCG GCGGGCAGGG  
1751 GCCAAACTTT CCCGGCGCGC GGAGGAGAAG AGACTGGGGA GGGAGGCAGA  
1801 GCCGAGGGGA ACGGCGTCGG GAGTGGCCGG ATGGAGGAAC TTGGGCGCGG  
1851 CGCGCGAGAA GTGGGACCCG GGTGCGGGGG CCCCAGGAGC GGGGCCAGGC  
1901 CCTCCCTGGG CTCGGGAGGC GCTTGGGAAG TTCTGTCCCC GCTGCCTGCG  
1951 CGTGGGGAGG ACCGAGGCC TTTTCGCCGG AGCGCGGGGC CGCGGCGCTC  
2001 ACCTGCGCCT TCTCGGGAGC CCCACCCGG CAGCATCCCG AAGGGAAGGT  
2051 CGGGCCCGGT GGGCGCGCTG CGGAGCGGAG CCTGGACTGG GGTCCCGCGC

FIGURE 12 (continued)

2101 GGCCTGGCC CTGCGGAGCG GAGCGGGAGG GGCAGAGGTG CTCGCCGGCG  
2151 GGACTGGGAG GGAGAAGGAC CTGCTCGACC TTGGACGCGG AGGTCATTTT  
2201 CCCAGCTCCG GGGTCTGGCC TCGCTAGCCA CCCCCCAA TCCGGAGCC  
2251 CCTTTCTTTC TGTTTCCTTC CTTCCCCTTT GGGCGCTTTT TTTGCTCCCG  
2301 CGGCCAGATG AACTTGGGGC GCTGTCCCTT CGGCTCCCCG AGCCGCATCC  
2351 TGTCTTGGTG GCTGCTGCTG GCCGGGAGGA GGCTGATGAA TACAGAGCCG  
2401 TGGAACAGGT CGTGCCGGAG ATGGAAACAG GAAAGCCTGT TGTTTTGTCTG  
2451 TCCCAG

FIGURE 13

1 GTGAGCCACC ACCGCGGCCG GTCCCTTCCT CCTTTAAAAA TTTTCTCCC  
51 AGTTCCCACT TTTTGTGGGT TAGAGGCATC TAAATTGAAT GAAAGTACCC  
101 TTTTGGGACT ACTGGGGAGG TGGGGGGATG TTCTCAGAAG GGAATTTTC  
151 TTTCTGGTCC TAATATCCAC CTAATTTTAA AAAGCAGGGC TCCTTATTAT  
201 TTTGTAAAGT TTACAATTAC ATCATTAGAT ACTTCCATGT CTCATATTTT  
251 ATTTTTCCTAA ACTCTTGGGG GAAATGAGTG GAGGGATGGA TGGAATAGAA  
301 AATAGTTTTT CCTCTTGGAG GCTGAGGGCC CAGTAGGGGT CAACAGTACA  
351 TTCAGCCCTC TCCTCACATA TTCTGTTCTA CCTACAAGTA CAGCAAGTAA  
401 AGCCAAATTT CTCATGCATG CAAATAAAGT TTTTGCATTT GGCCAGTCGG  
451 TCCAGTTCTC CTGTCAGCTT CCTTCCCCAC TCTGCCTCTG TTCATTAATC  
501 CCCCCCTTCC CGGTACCTAA ACCCTCCACC TAACCCAGCC CTTTCTTCCA  
551 CTTCCGGCTA CTAGCCTCTC TCGCCTATCC ACTATCCTCA CACTCAGCAT  
601 CCCCTGTCTG TACGAGATTA AGGAGCTCTG CCGTCCGCAG GGCCTGGGTT  
651 AGCGTGAATC TAAGCCAGAG CTCCCGGGTG GGGGTGGGGG TAGGGGTGGG  
701 GGTGGTCCCA GAGGTAGGGC GAGGAGGTGG GAAGCGTATT CCCTTCACTG  
751 GTGATCTCAA CGTAGATTTG CCCGGAGTTC TCTTGCAAGA GAGCTGGCAG  
801 GTTTTACTAT TTCCCAATCG TTTACTCGCC AAGCTCTCGG GTCCACGCGC  
851 CGCGGGGATG CGCCCTGCAA GCTGAAACTT CATTCAAAGC AAGGCGGCCC  
901 ACGAGGTTGG GCTTAGGGGA TCTGGATGAC CTCCAGGCCA CTTCTTTTCT  
951 CTCTGCGCCC TTCCCCCACT CTTCCAACCA CCTTCGCTGT AAACAAAAC  
1001 GTCCCCCCCCG GGCGGAGAGA GGTCGCGCTC TTTCGCACAC TCCCTCGCCA

FIGURE 13 (continued)

1051 AGGGTTAATT TCTCAAATCG CACGAGGGGG AGGAGATTTC CCTGTAGACG  
1101 AGTAAAAAGG GTGATGGACA AACGTGCGGG CACTAAGACC GCAAGGCATT  
1151 CATTTCTCTC TACGGTGGAT GCGGACGCCG GGAGGAGGAG AGCCCCAGAG  
1201 AGAGGAGCTG GGAGCGGAGG CGCAGGCAAT GCTCAGCCCT GGATGTAGCT  
1251 GAGAGGCTGG GAGAAGAGAC GACCGCTGGA GACCGAGCGG CGTGGGGAAG  
1301 ACCTAGGGGG GTGGGTGGGG GAAGCAGACA GGAGAACACT CGAAATCAAG  
1351 CGCTTTACAG ATTATTTTAT TTTGTATAGA GAACACGTAG CGACTCCGAA  
1401 GATCAGCCCC A

FIGURE 14

5' ATG GTC ACA GCC ATG AAT GTC TCA CAT GAA GTA AAT CAG CTG TTC CAG CCC TAT  
 M V T A M N V S H E V N Q L F Q P Y

30  
 60 AAC TTC GAG CTG TCC AAG GAC ATG AGG CCC TTT TTC GAG GAG TAT TGG GCA ACC  
 N F E L S K D M R P F F E E Y W A T

90  
 120 TCA TTC CCC ATA GCC CTG ATC TAC CTG GTT CTC ATC GCT GTG GGG CAG AAC TAC  
 S F P I A L I Y L V L I A V G Q N Y

150  
 180 ATG AAG GAA CGC AAG GGC TTC AAC CTG CAA GGG CCT CTC ATC CTC TGG TCC TTC  
 M K E R K G F N L Q G P L I L W S F

210  
 240 TGC CTT GCA ATC TTC AGT ATC CTG GGG GCA GTG AGG ATG TGG GGC ATT ATG GGG  
 C L A I F S I L G A V R M W G I M G

270  
 300 ACT GTG CTA CTT ACC GGG GGC CTA AAG CAA ACC GTG TGC TTC ATC AAC TTC ATC  
 T V L L T G G L K Q T V C F I N F I

330  
 360 GAT AAT TCC ACA GTC AAA TTC TGG TCC TGG GTC TTT CTT CTC AGC AAG GTC ATA  
 D N S T V K F W S W V F L L S K V I

390  
 420 GAA CTC GGA GAC ACA GCC TTC ATC ATC CTG CGT AAG CGG CCA CTC ATC TTT ATT  
 E L G D T A F I I L R K R P L I F I

450  
 480 CAC TGG TAC CAC CAC AGC ACA GTG CTC GTG TAC ACA AGC TTT GGA TAC AAG AAC  
 H W Y H H S T V L V Y T S F G Y K N

510  
 540 AAA GTG CCT GCA GGA GGC TGG TTC GTC ACC ATG AAC TTT GGT GTT CAT GCC ATC  
 K V P A G G W F V T M N F G V H A I

570  
 ATG TAC ACC TAC TAC ACT CTG AAG GCT GCC AAC GTG AAG CCC CCC AAG ATG CTG  
 M Y T Y Y T L K A A N V K P P K M L

FIGURE 14 (continued)

600  
 CCC ATG CTC ATC ACC AGC CTG CAG ATC TTG CAG ATG TTT GTA GGA GCC ATC GTC  
 P M L I T S L Q I L Q M F V G A I V

660  
 AGC ATC CTC ACG TAC ATC TGG AGG CAG GAT CAG GGA TGC CAC ACC ACG ATG GAA  
 S I L T Y I W R Q D Q G C H T T M E

720  
 CAC TTA TTC TGG TCC TTC ATC TTG TAT ATG ACC TAT TTC ATC CTC TTT GCC CAC  
 H L F W S F I L Y M T Y F I L F A H

780  
 TTC TTC TGC CAG ACC TAC ATC AGG CCC AAG GTC AAA GCC AAG ACC AAG AGC CAG  
 F F C Q T Y I R P K V K A K T K S Q

810

TGA 3'

\*

FIGURE 15

1 GATTAGCTGT CAGGCTATAT ATGGAGCCAT CAGGAACCAC TGAAGGTTTT  
51 TTTTTTTTTT TTTTTTTTTG AGACGGAGTC TCACTCTGTC ACCCAGGCTG  
101 GAGTGCAGTG GCACGATCTC TGCTCACTGC AAGCTCTGCC TCCCAGGTTC  
151 ACGCCATTCT CCTGCCTCAG CCTCCCGAGT AGCTGGGACT ACAGGCGCCT  
201 GCCACCACGC CCGGCTAATT TTTTGTATTT TTTAGTAGAG ACGGGGTTTG  
251 ACGGTGTTAG CCAGGATGGT CTCGATCTCC TGACCTCATG ATCTGCCCCG  
301 CTCGGCCTCC CAAGGTGCTG GGATTACAGG CGTGAACCAC CGTGCCCGGC  
351 CGAACCCTG AAGGTTTTTA AGCAGGAAAG CAGAGCTGTT TTCTGGATGA  
401 GCAAACAGAA AGTAGTGGTT TTCCAAGTAC AGTCTGAGAC AACCTATAGG  
451 ACCAGAATCT CTGCAGTTGA GGCTCAGGAA TCTGGTAATC AGCCAGGTAT  
501 AGGAACTCTT TTCTGATTGC AATGCAGTGA AGAGCAGAAG CACTGTATTA  
551 GAGAAAGAGG CAGTGCAACC AGGTAACGTG ACCAGGTGAG AAGTGATGAG  
601 GTACAGAGAC AAAGAGATGC ACTTTTGAGT CACTTAGATG GCACTGATAG  
651 GACTTCCACT ACACCCTCGC ATAGACAGTG GCTGAGGTTC AGGAAATAGA  
701 GCTGGGGTTC CTACTTGAT CCTCTGGCTC TAGAGCTTTA CTGCACATAG  
751 CCATTTATAC CCACATCTTG ATTTTAATTA TTTTATATCT ATGTTTCTTA  
801 GCACTTTTTG CAAATTTCCA CCTTATCTCA AACTGCCCTC AAGCCTTGTA  
851 TTTCTCCTTC GCTTTCATAA AACCTAGGAA AGAAATAAGG GACAGCCAAG  
901 TAAAACTTTT AAAAGTTTTA GAACATTTAT TTCTTTGGGG CTGGTTACAC  
951 AGGCGAGAAA GAAGTAGATT TGGTTAGGGA GAGAAAACAA CAGGCCTTGG  
1001 GGAGATACAC TGGCTCTCCC CCTCCCTAAA CCCTAAGAGG CCTCCAGGAA

FIGURE 15 (continued)

1051 ACCTGAAGAC AATAATTCCA GAAGCCCAGA GGGTGACCCC ATTCCTCTC  
1101 TCCATGGTTA TTACTGTCAG TCTGGAGCAG TTCAGGAATT CAGGAAACTA  
1151 TAAAGAAACC ACAACAGCCT CAACAACCCA AACATCAACA TCAACAACCT  
1201 CAACAATAAA ACTCCTTAAA ATTCATCTCC TTCCACCCAC TCACAACCGC  
1251 AGACTCGAAG CTAGGAGGTG GAAGGGACTA CAGAAGCTCT GCGTTGCCCA  
1301 GGTTAGTATT TGCTCATCAC AGGCCTGGGT TTCCCAGGAT CTCAGGGAGC  
1351 CTGGAAACTG ACGCCTCCAT TTCTGGGTGG GAGCACCAA GCCTAAGGAC  
1401 ACCTTTCCTC TCTCTTCACT GCTAAGCAGG TCAAGATTAA AGCAAACCGA  
1451 GGCAAAGGCC ACGGTTGACA GTTCCAAGGG AACCCGCAAG GCCGCACAGG  
1501 ATGGGGTGGA CGTTTTACGG GAGAAAAGGC TGGGGAAGTG GGCGGGCGAT  
1551 GGCCTACGAC GGGACTTGGG GCGGGGTGTG CGAAACGCCT GGCAGGCGGG  
1601 CCCTTGAGTA TGACCAATCA GAATGCGGAC TGCGTCCCAG GGGCGGAGCA  
1651 GAGGCGTATC TTGGTCGAGA TTGGATAGCG GCGGGGCGCA GGAAAGAGGT  
1701 CGCGCCAGCC CGGGCAGGCA GCTTTGCAAG TCCGCGTTAT ATATCGCAGT  
1751 GGCTGCGCCC GGGATAGCTG GCTGCGCCGC CGCGCACATG CCTAGGTTCG  
1801 ACGCCCTCCT CCCTTTGCCC AGGAGTTCCT TCTGTCCCGG CTCTGTTCCG  
1851 TCTCGCCCCG AGGTTCACGC CATCCTCGGA GCCCCAGCCT TTCACCCAGC  
1901 GCCTCCAAGC TTTGGACCTT GACTTCTGCA AACTAG



FIGURE 16

```

5'   ATG GGG CTC CTG GAC TCG GAG CCG GGT AGT GTC CTA AAC GTA GTG TCC ACG GCA
      M  G  L  L  D  S  E  P  G  S  V  L  N  V  V  S  T  A

                                     30

      CTC AAC GAC ACG GTA GAG TTC TAC CGC TGG ACC TGG TCC ATC GCA GAT AAG CGT
      L  N  D  T  V  E  F  Y  R  W  T  W  S  I  A  D  K  R

      60                                     90

      GTG GAA AAT TGG CCT CTG ATG CAG TCT CCT TGG CCT ACA CTA AGT ATA AGC ACT
      V  E  N  W  P  L  M  Q  S  P  W  P  T  L  S  I  S  T

      120                                     150

      CTT TAT CTC CTG TTT GTG TGG CTG GGT CCA AAA TGG ATG AAG GAC CGA GAA CCT
      L  Y  L  L  F  V  W  L  G  P  K  W  M  K  D  R  E  P

      180                                     210

      TTT CAG ATG CGT CTA GTG CTC ATT ATC TAT AAT TTT GGG ATG GTT TTG CTT AAC
      F  Q  M  R  L  V  L  I  I  Y  N  F  G  M  V  L  L  N

      240                                     270

      CTC TTT ATC TTC AGA GAG TTA TTC ATG GGA TCA TAT AAT GCG GGA TAT AGC TAT
      L  F  I  F  R  E  L  F  M  G  S  Y  N  A  G  Y  S  Y

      300

      ATT TGC CAG AGT GTG GAT TAT TCT AAT AAT GTT CAT GAA GTC AGG ATA GCT GCT
      I  C  Q  S  V  D  Y  S  N  N  V  H  E  V  R  I  A  A

      330                                     360

      GCT CTG TGG TGG TAC TTT GTA TCT AAA GGA GTT GAG TAT TTG GAC ACA GTG TTT
      A  L  W  W  Y  F  V  S  K  G  V  E  Y  L  D  T  V  F

      390                                     420

      TTT ATT CTG AGA AAG AAA AAC AAC CAA GTT TCT TTC CTT CAT GTG TAT CAT CAC
      F  I  L  R  K  K  N  N  Q  V  S  F  L  H  V  Y  H  H

      450                                     480

      TGT ACG ATG TTT ACC TTG TGG TGG ATT GGA ATT AAG TGG GTT GCA GGA GGA CAA
      C  T  M  F  T  L  W  W  I  G  I  K  W  V  A  G  G  Q

      510                                     540

      GCA TTT TTT GGA GCC CAG TTG AAT TCC TTT ATC CAT GTG ATT ATG TAC TCA TAC
      A  F  F  G  A  Q  L  N  S  F  I  H  V  I  M  Y  S  Y

```

FIGURE 16 (continued)

600  
 TAT GGG TTA ACT GCA TTT GGC CCA TGG ATT CAG AAA TAT CTT TGG TGG AAA CGA  
 Y G L T A F G P W I Q K Y L W W K R

660  
 TAC CTG ACT ATG TTG CAA CTG ATT CAA TTC CAT GTG ACC ATT GGG CAC ACG GCA  
 Y L T M L Q L I Q F H V T I G H T A

720  
 CTG TCT CTT TAC ACT GAC TGC CCC TTC CCC AAA TGG ATG CAC TGG GCT CTA ATT  
 L S L Y T D C P F P K W M H W A L I

780  
 GCC TAT GCA ATC AGC TTC ATA TTT CTC TTT CTT AAC TTC TAC ATT CGG ACA TAC  
 A Y A I S F I F L F L N F Y I R T Y

840  
 AAA GAG CCT AAG AAA CCA AAA GCT GGA AAA ACA GCC ATG AAT GGT ATT TCA GCA  
 K E P K K P K A G K T A M N G I S A

870  
 AAT GGT GTG AGC AAA TCA GAA AAA CAA CTC ATG ATA GAA AAT GGA AAA AAG CAG  
 N G V S K S E K Q L M I E N G K K Q

930  
 AAA AAT GGA AAA GCA AAA GGA GAT TAA 3'  
 K N G K A K G D \*

FIGURE 17

1 GGAAATACCT GAAGCTGTTT TAACAATTTT TCCTTGTATT AAGTATTATG  
51 CTGCAGTTTT GCGTGTGTGA ATGGAAGTAT GGGTAGAGAT CTGTTCTCCC  
101 TAAAAACTCC AGGATTCCAC AATATAGAAA TAGTAATCAA ATTTTATAGGT  
151 GAAGCTCGAA CTAATCCGAA CTTTGTTAGA TCATCACTGT AAATGAATGG  
201 GTATTTATCC ACTCCCTAAA TGAAGAGACT TGACTGGATT TCTTTTTTTT  
251 ATATAGCTAC TAGAATCTGT TACACATAAT TTAGGATTGA GACTTGAGAA  
301 ATTGTCATTC CAATCCAGAA AACTTTAGAT TTGCAAATAT ATTTGACAAA  
351 TTAATAAATT AACATTTTAT TTGGTTAATT TCAAGAATAG GGCATTTAAA  
401 GAAGTCTGTG TTTGCTTTAG TTCGGCAATA AAGTTCCTGC CACTCACAAT  
451 AATCCTTATT ATTCTCTGAA AGACATGTTA TATTTTGTGTC ATCATAAATA  
501 TTTATTAATT ACTGTTTATA GCACTGGGTT AGGTACTCAT CAAGCAACCA  
551 AAAATAATTC TTACCATCTA GGATGCTTCC AATATAAAAT ATAGACAATA  
601 TATAACCAGG TCAATTGGGA AATAGATCAT TTCAGTATGA TAAAAGATAG  
651 TATTCACATT AACAGTGTGA AAGGGCAGGA ACAATAAGAC ACTTGACTCA  
701 CTGGTCTTTA AAATGTAGCA TCCAAAATGA GCAAGTGGAG AAAAGGTTAA  
751 ACAAGTAGGT GACACATTTA AAAACAAGT AGATGAAAGG ACTATTCTCA  
801 AAAATCTTGT TTTATGTGAG AAACCATCAA ATTATGAATT CCAAGTACTG  
851 TATTTTTTTT ACTTTTCAAG GGTAGGCTCT CCTATACCTT ATCTAAACAA  
901 TTTTTCAAAA TAGCCACAAT TACTTTGTTT TCCTCTCTAC ACTAAATTGC  
951 CCTTTGCCTC TTGAGCGATT ATCTTTTTCA GATTCACCTC AACTTCTTCA  
1001 GGTTC AAGCG GACTTCACCT GTAAGCCCCT CTCGGTTCTC CCTCTTCTCT

FIGURE 17 (continued)

1051 GAACTACTAA TGGCCTAATT TAGCACAATT ATATTGCTTT GTTCATTCCA  
1101 TGTATAGTAA AAGAGTCTAC AAAACACATG CAAGCATTCA TGCAATTATA  
1151 TGTTGATTTG TTCATGGGTC GACCCCAAAG TCTATTCTCC ATCGCTGAAG  
1201 CATGGAAGAC AAATACCCTT CACTTCTTCA GAGGCATAAC ACATGCACTT  
1251 CTCTTGTCAT GGTGACAGGC ATGTGCTGGT GGAGGTCAAA GAAACAGGAA  
1301 CACAAGTGAA ATCGAGGTGA GTGTCAGGTA AGGACCAAAG CACCACGCCT  
1351 ACCTCATCTT TGCCACAGA ACACCCATTC TTCCCGTGTC CTGTTTCCCA  
1401 GGACGTATCC GGGGCGGATA AGAAATCACC CGTGGGGAGG CGGTGAACTC  
1451 CTCCGCAGGG GCCGATGCCC GGGACAGGGG CGGGGAAGGC TAATGAGGCG  
1501 ACTTG TGCGG GGAGGGGCCA AGGAGGAGCC CAGGTGTCCC GCTCCCGCTC  
1551 GACGGCGCGC GCCTGCGCGA GCCCAGTTGG CGTCGCACCC TTGAGCGCAG  
1601 CATCCCTACG CCAGCGAGTC CCAATACTAG GGAGGGAGGG AGGGAGGAGG  
1651 GGCGGCCGGC CCCCCGCCCC CGCGCGCGGC CACGTGACGC CGGCTGAGGA  
1701 GATTGGAGGG GCGGCTGCGC GAGGCTGCAG ACTGGTGCAG CGCACTGTGC  
1751 TGGCGGCTGG GCCTCCTCCA CCTCCTCGTC TTTCTCCCGG GAACCTTGAC  
1801 GACGCCTTCC GCTTGGCCCT GCCTTCTGCC GCATCCCCGC CGCCGCGGCG  
1851 CCTTGAGGAG CAGGAGAAGA CGCAGCCGGG CCGCCGCCGT TAGAGGGGTT  
1901 CCCGGCCGCC GCTCGCCCCG TCGGCCGCCA CCGCCTCCGG GGTGAGCCCT  
1951 CTCTCTGGGT CTCCGCTTTC TCCTGCCGCC AGCGCCCGCT CATCGCCGCG

FIGURE 18

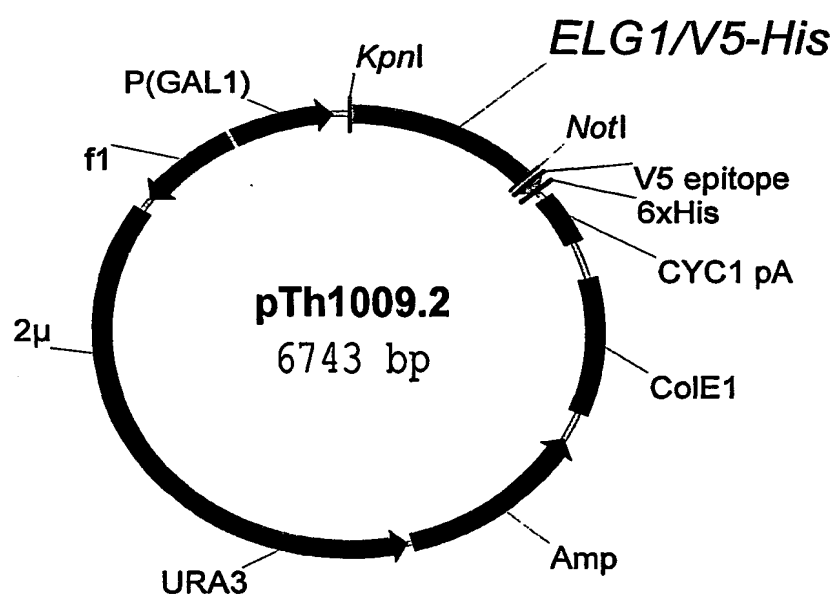


FIGURE 19

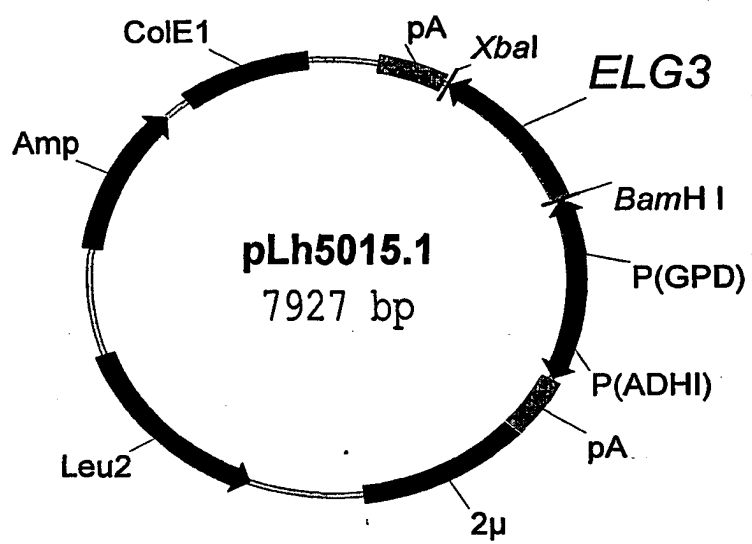


FIGURE 20

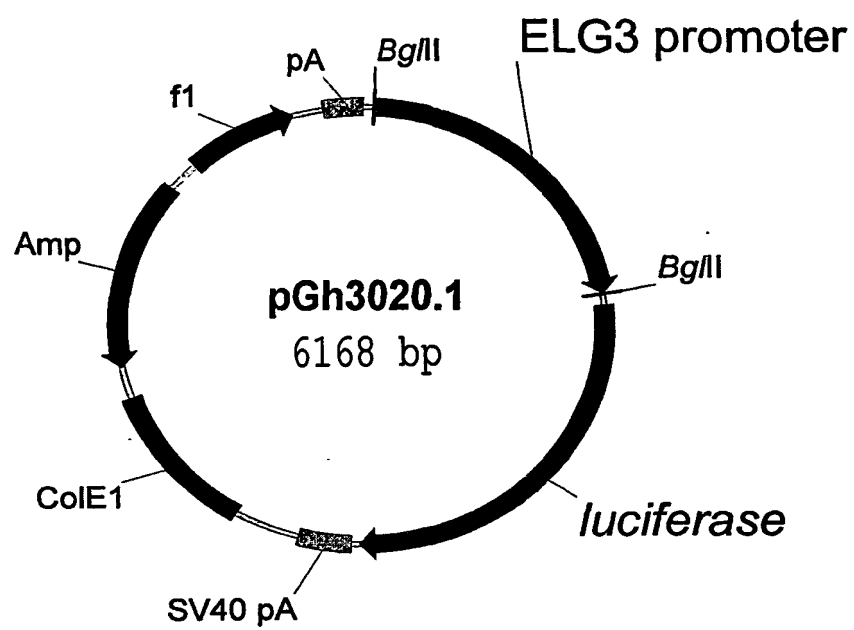


FIGURE 21

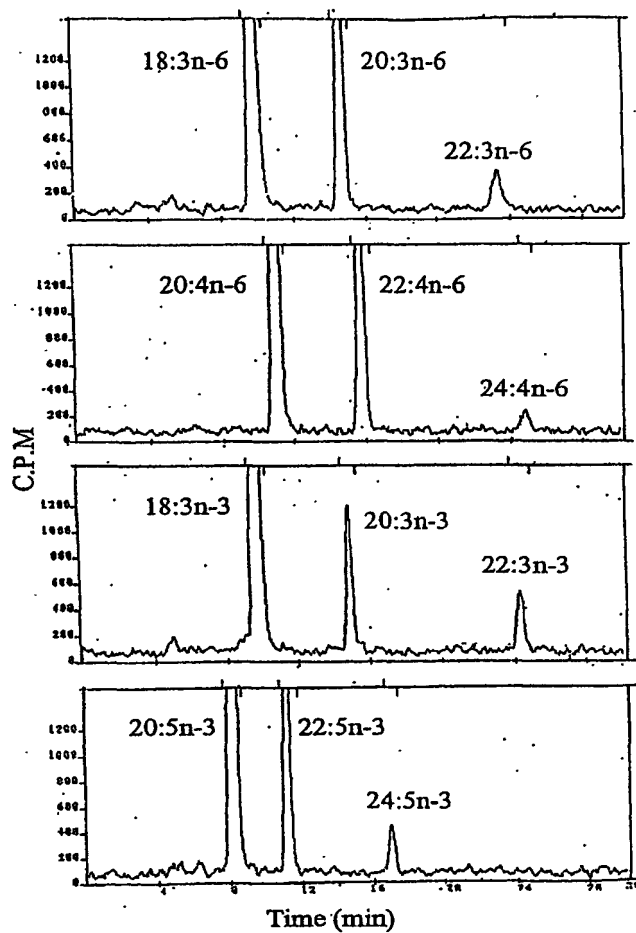




FIGURE 22

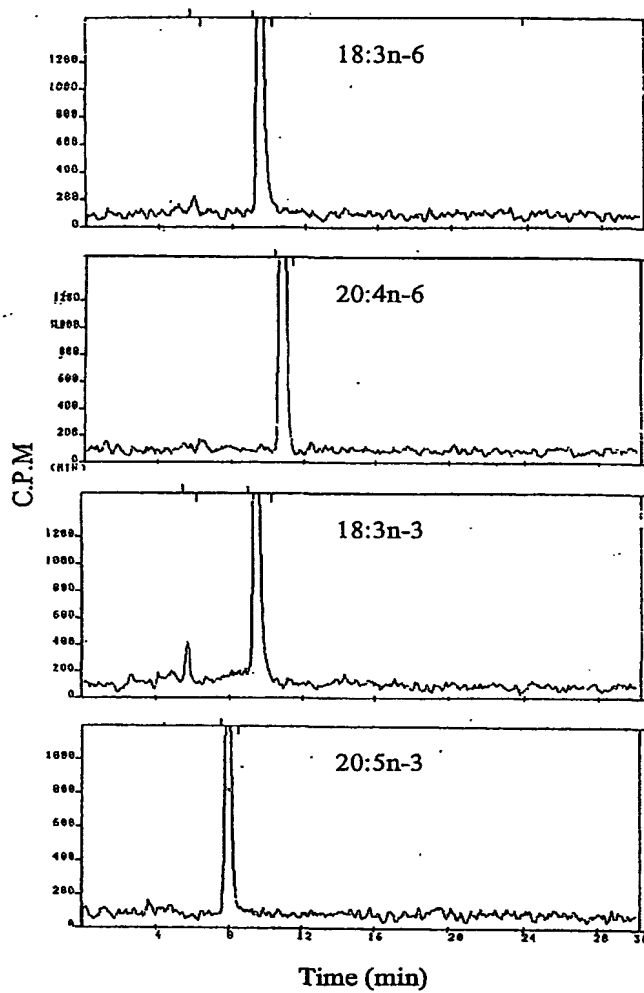


FIGURE 23

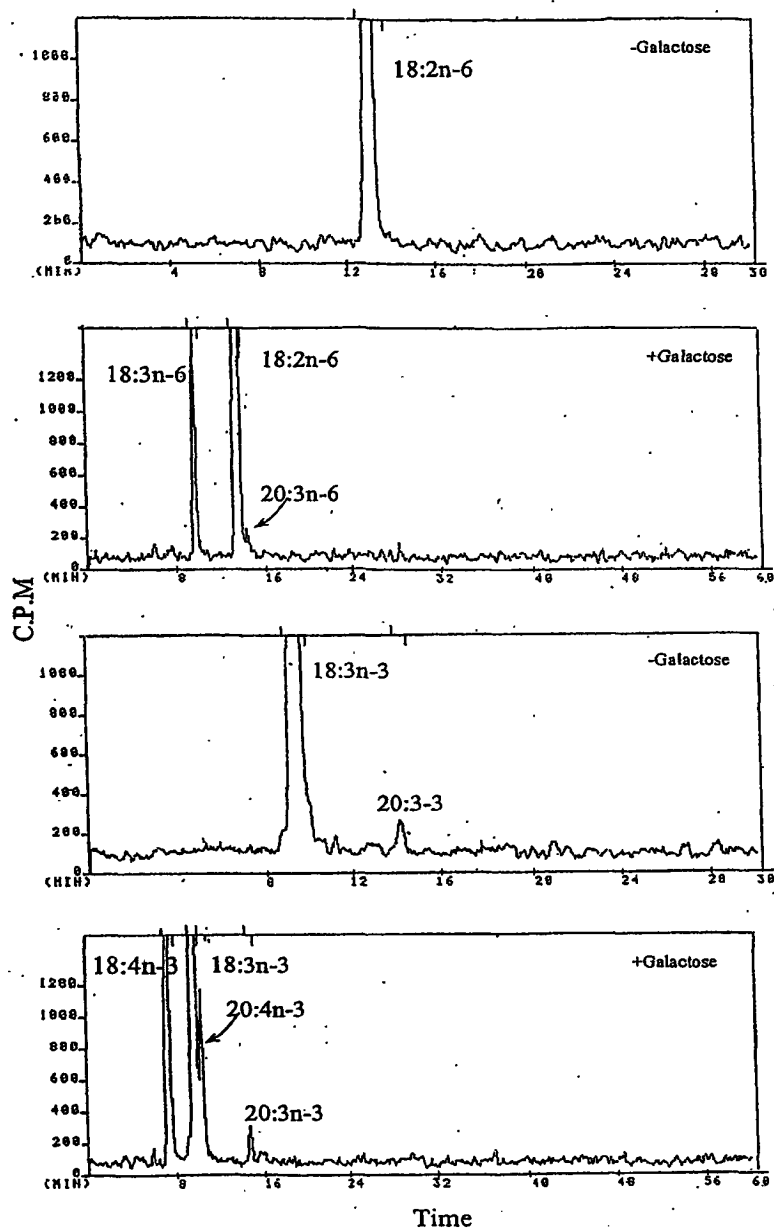


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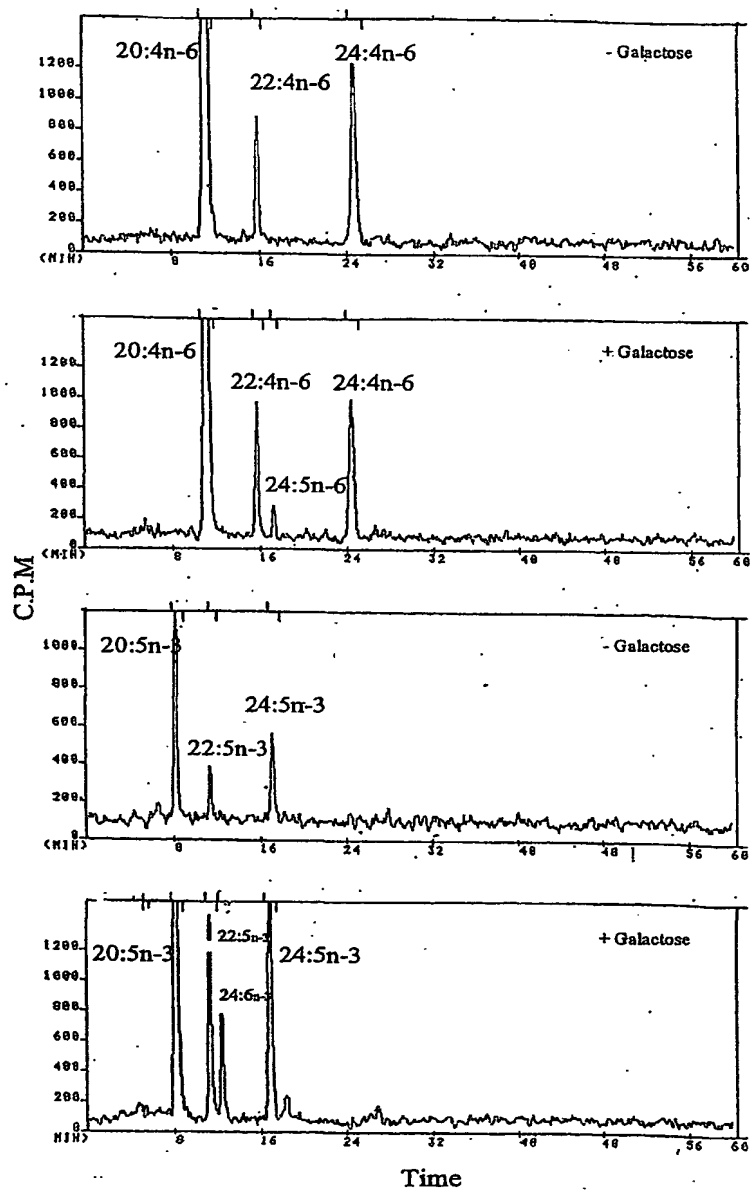


FIGURE 25

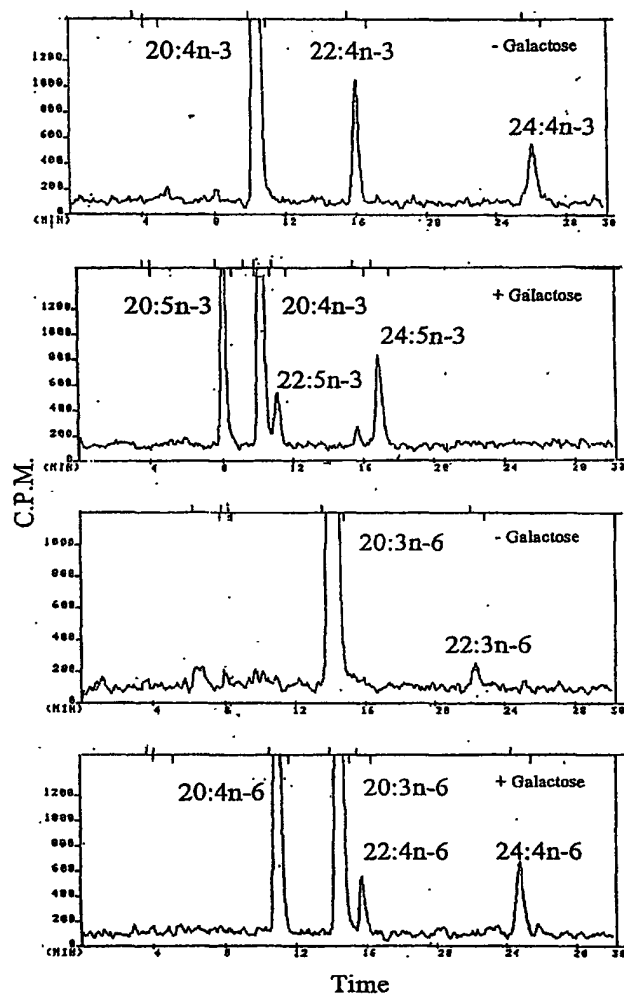
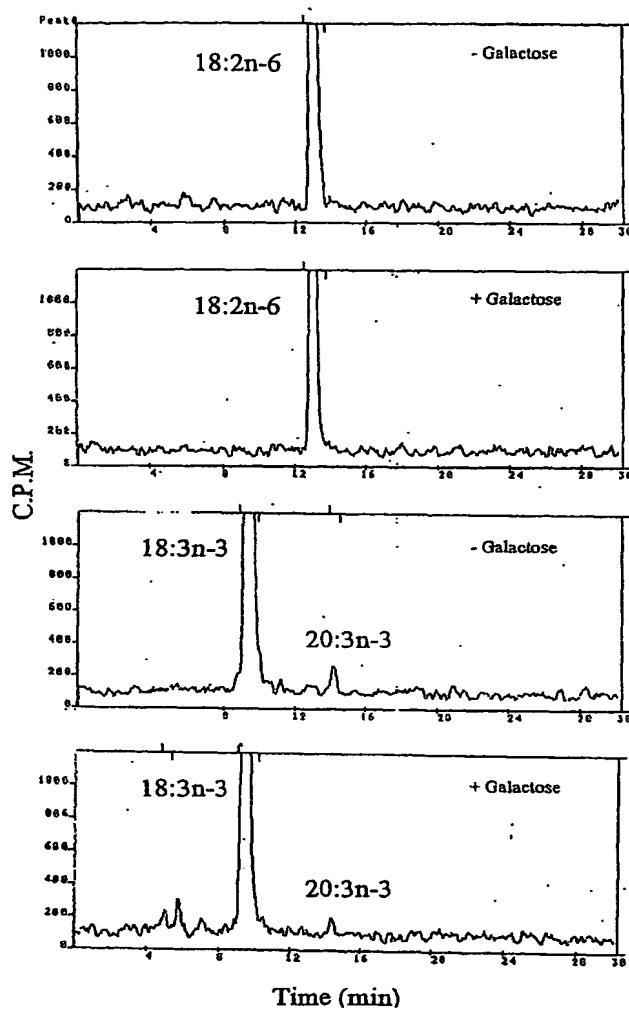


FIGURE 26



## SEQUENCE LISTING

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 Knickle, Leah C  
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 Allen, Stephen J  
 Ponton, Andre  
 De Antueno, Roberto J  
 Jenkins, D K  
 Nwaka, Solomon O  
 Goldberg, Y Paul

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&lt;213&gt; Artificial Sequence

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&lt;223&gt; Description of Artificial Sequence:Primer

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<210> 24

<211> 45

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<211> 44

<212> DNA

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<210> 28  
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<210> 51

<211> 28

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<210> 53

<211> 28

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28

<210> 54

<211> 31

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<223> Description of Artificial Sequence:Primer

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<210> 55

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<212> DNA

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